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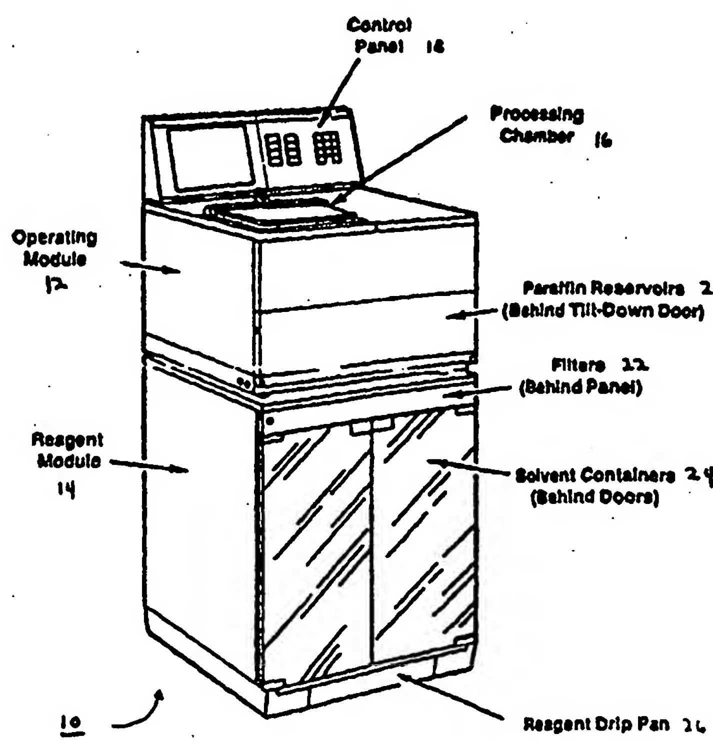
(54) Title: METHOD AND APPARATUS FOR AUTOMATED REPROCESSING OF TISSUE SAMPLES


(57) Abstract

A method and apparatus of automatically reprocessing a specimen for microscopic examination is disclosed. Processing of a specimen for microscopic examination involves fixation of the specimen and preparation of the embedded specimen from the fixed specimen. There are instances where, once a specimen has been processed, it is necessary to reprocess the specimen due to contamination of reagents during processing or inadequate fixation. The system automatically reprocesses a specimen by removing residual embedding material from the specimen with a clearing agent, removing the clearing agent with a dehydrating agent, and removing the dehydrating agent with an aqueous fluid.

# METHOD AND APPARATUS FOR AUTOMATED REPROCESSING OF TISSUE SAMPLES



## BACKGROUND OF THE INVENTION

### A. Field of the Invention

This invention relates generally to the fields of histology and cytology, and more particularly relates to a method and apparatus for reprocessing and processing a specimen.

### B. Description of Related Art

Microscopic examination of specimen samples typically involves examining a slice or a cross-section of the sample. In order to obtain a cross-section, the specimen sample undergoes a process to infiltrate the specimen with a paraffin wax or a wax substitute. Thereafter, the block is embedded and sliced into sections using a microtome.

The method of processing the specimen involves fixation of the specimen and preparation of the infiltrated specimen from the fixed specimen. Fixation of the specimen typically involves immersion, subjecting or exposure of the specimen in a

fixing agent, such as formalin. Preparation of the infiltrated specimen from the fixed specimen is typically a time-consuming, multi-step process requiring dehydration of the fixed specimen with a dehydrant (such as alcohol), clearing of the dehydrant with a suitable clearant (a typical solvent is xylene), and infiltration of the specimen with an infiltrating medium, such as paraffin wax. In addition, the dehydration and clearing steps typically require immersion, subjecting or exposure of the specimen in a graded series of reagents for comparatively long periods of time. The time required for tissue preparation may be on the order of 8 to 12 hours. Examples of tissue preparation are in U.S. Patent No. 3,961,097 entitled "Method of Preparing Tissue for Microscopic Examination" and U.S. Patent No. 4,656,047 entitled "Rapid Method for Cell Block Preparation," both of which are hereby incorporated by reference in their entirety.

Different types of specimens, such as any organelle, cell, cell suspension, tissue section, or tissue specimen, may be infiltrated with a paraffin medium for examination. However, different types of specimens may require different types of procedures to be processed properly. In addition, there may be instances where the specimen may be processed incorrectly, due to contamination of reagents during processing or inadequate fixation. It is typically not until after the specimen has been embedded and sliced that it can be determined whether the specimen has been properly processed. At that point, there are two options: obtain another specimen or reprocess the embedded specimen. If one chooses to reprocess the sample, this involves sequentially immersing, exposing or subjecting the specimen with a series of reagents under controlled conditions. However, this process is very time-consuming and requires a technician to manually proceed through each of the reprocessing steps.

Further, there are instances where a slice or a cross-section of a specimen, after being processed, will be reprocessed for analysis. One instance is ploidy analysis in which tissue sections are cut from the paraffin block, wrapped in a permeable material and reprocessed. The reprocessing steps remove the paraffin using a clearing agent, remove the clearing agent using a dehydrant and remove the dehydrant using an aqueous medium. Nuclei from the specimen are then prepared for DNA analysis using a fluorescent compound.

## SUMMARY OF THE INVENTION

In accordance with a first aspect of the invention, an apparatus for automatically reprocessing a specimen from an infiltrated medium to an aqueous fluid is provided. The apparatus has a processing chamber for holding a specimen, means for regulating flow of fluid to the processing chamber, at least one container of a clearant agent, at least one container of a dehydrant agent and at least one container of an aqueous fluid, the containers of clearant, dehydrant and aqueous fluid being connected to the processing chamber via means for regulating flow of fluid to the processing chamber, and a control device having a processor and a memory device, the processor controlling the means for regulating flow of fluid in order to automatically and sequentially connect the processing chamber with the container of clearant agent, the container of dehydrant agent and the container of aqueous solution in order to reprocess the specimen.

In accordance with a second aspect of the invention, a method for automatically reprocessing a specimen using a specimen reprocessing machine having processor for controlling the exposure of the specimen to a clearing agent, a dehydrating agent and an aqueous fluid is provided. The method includes the step of providing the specimen which is infiltrated with an infiltrating medium, indicating to the specimen reprocessing machine that the specimen is to be reprocessed, exposing the specimen to a clearing agent via the processor to remove the infiltrating medium from the specimen, exposing the specimen to a dehydrating agent via the processor to remove the clearing agent, and exposing the specimen to an aqueous fluid via the processor to remove the dehydrating agent from the specimen.

Accordingly, a goal is to process and reprocess specimens for microscopic

examination. These and other objects, features, and advantages of the present invention are discussed or apparent in the following detailed description.

## BRIEF DESCRIPTION OF THE DRAWINGS

A presently preferred embodiment of the present invention is described herein with reference to the drawings wherein:

FIG. 1 is a front perspective view of the processing and reprocessing system;

FIG. 2a is block diagram of the Operating Module of the processing and reprocessing system;

FIG. 2b is block diagram of the Reagent Module of the processing and reprocessing system;

FIG. 3 is a block diagram of the pressure modifier, float valve and processing chamber in the Operating Module and Reagent Module of Figures 2a and 2b;

FIG. 4 is a front view of the Reagent Module of Figure 1 with the doors removed;

FIG. 5 is a flow chart of the processing of a specimen;

FIG. 6a is a flow chart of the reprocessing of a specimen until introduction of an aqueous fluid in the specimen and processing of the specimen; and

FIG. 6b is a flow chart of the reprocessing of a specimen, until the step as indicated by the operator, and processing of the specimen.

## DETAILED DESCRIPTION OF PREFERRED AND ALTERNATIVE EMBODIMENTS OF THE INVENTION

The processing and reprocessing of tissue is accomplished by sequentially putting the specimen (such as any organelle, cell, cell suspension, tissue section, or tissue specimen) to be processed or reprocessed in contact with, or immersed in, a series of reagents under controlled conditions. The reagents may be divided into three

types: paraffin, solvents and aqueous solvents. The conditions that can be controlled while the tissue is in contact with a reagent can be any combination of heat, pressure, vacuum and agitation.

Referring to Figure 1, there is shown one example of a tissue processing and reprocessing system 10. The tissue processing and reprocessing system may consist of two major components: an Operating Module 12 and a Reagent Module 14. The Operating Module and Reagent Module can be placed side-by-side on a benchtop or stacked for a floor mounted configuration, as shown in Figure 1. The specimen is placed in a processing chamber 16, and reagents are sequentially put into the processing chamber 16 from the solvent containers 24, with excess reagents being collected in the reagent drip pan 26 in case of a malfunction. Paraffin is also introduced into the processing chamber 16 with paraffin reservoirs 20. In this apparatus, the control panel 18 indicates the operation of the system 10 and allows for control of the heat, pressure, vacuum and agitation, which affect the processing chamber 16.

An alternative method of putting the specimen in contact with, or immersed in, the reagents is to have each reagent contained in a separate container, and have a mechanical device, such as a robotic arm and controls, to move the specimen from container to container. With this method, the systems for controlling the heat, pressure, vacuum and agitation can, in any combination, be attached to the individual reagent containers or the device for moving the specimen.

Referring to Figure 2a, there is shown a block diagram of the Operating Module 12. The Operating Module 12 houses the processing chamber 16, the control device 28, Input/Output device 30, and the paraffin oven 32 with three reservoirs 20.

The processing chamber 16 inside the Operating Module 12 connects through tubing to valve 34, such as a rotary valve (or other means for regulating flow of a fluid) to the paraffin reservoirs, and through tubing to the reagents in the Reagent Module. In an alternate embodiment, the means for regulating flow of fluid from the paraffin reservoirs to the processing chamber may be performed by any valve, flap, lid, or plug. The processing chamber has an agitator 36, used when the specimen and reagent require stirring. The agitator 36 may be in the form of a rotating stirring device, a recirculating pump, or any other device that causes the reagent to move with respect to the tissue or the tissue to move with respect to the reagent. In addition, the processing chamber has a pressure sensor 38, used to indicate the pressure in the processing chamber 16 to the pressure modifier 44. As described subsequently, the pressure modifier 44 may be accomplished through mechanical means by applying direct mechanical force to the processing chamber through an aneroid, diaphragm, or other mechanical device. The pressure may also be changed by applying pneumatic pressure or vacuum to the processing chamber (*e.g.*, a compressor (air pump) in the system or an external source of vacuum and/or pressure). This may also be accomplished with a mechanical regulator or by cycling the sources of vacuum or pressure on and off.

The Operating Module 12 also includes the paraffin oven 32. Processing and reprocessing of tissue may include the use of an infiltrating medium such as paraffin. The paraffin is stored in a temperature-controlled container in order to keep the paraffin in a liquid state. The temperature of the paraffin reservoirs 20 can be controlled by applying heat directly to the individual containers or by having the paraffin container(s) in a temperature-controlled chamber (such as an oven 32). The

oven 32 maintains the paraffin in a liquid state so the system can draw the paraffin into the processing chamber 16, allowing it to penetrate the samples. The processing chamber 16 connects to the paraffin reservoirs through a heated rotary valve 34, which facilitates paraffin selection. At the proper time in the processing and reprocessing program, the rotary valve 34 permits paraffin from the selected reservoir to flow into the processing chamber, drawn in under vacuum. During the drain cycle, the valve also selects the proper reservoir for the chamber to empty into.

Referring to Figure 2b, there is shown a block diagram of the Reagent Module 14. Processing and reprocessing may require the use of reagents. The Reagent Module 14 contains reagent containers 40 and is connected to the processing chamber 16 in the Operating Module via a solvent/purge line. In one embodiment, the Reagent Module 14 contains twelve reagent containers: ten solvent containers and two purge containers (as shown in Figure 4). The storage temperature of the solvents typically do not need to be controlled and are therefore stored at room temperature. In this arrangement, there is a means for selecting the specific reagent container 40 to move reagents into the processing chamber 16. In one embodiment, the specific reagent container is selected via a set of two valves, one valve 42 (which is set by the processor 54) in the Reagent Module and the second valve 34 (which is set by the processor 54) in the Operating Module. The valve 42 acts as a means for regulating the flow of fluid (which in a preferred embodiment is a liquid and in alternate embodiments may include a liquid, gas or both liquid and gas) between the container 40 and the processing chamber. The valve 42 selects which solvent container connects to the fluid line going to the Operating Module. Thereafter, valve 34 selects which of the paraffin lines or solvent/purge line is connected to the processing

chamber. At the proper time in the processing or reprocessing program, the rotary valves 34, 42 (as set by the processor 54) permit only one solvent to flow through the line into the processing chamber, drawn in under vacuum. During the drain cycle, the processor 54 selects the proper setting of valves 34, 42 (as set by the processor 54) for the proper station in order to permit the chamber to empty under pressure. In an alternative embodiment, a valve or other means for regulating the flow of fluid may be mounted on each individual reagent container and connected to a common manifold which connects to the processing chamber. In another embodiment, other means for regulating the flow of liquid between the containers and the processing chamber include any valve, flap, lid, or plug.

The Reagent Module 14 also has another line to the Operating Module 12 that modifies the pressure in the processing chamber 16. The pressure is modified in the tissue processing and reprocessing system via a pressure modifier 44. As shown in more detail in Figure 3, the pressure modifier 44 serves as a means for introducing and extracting reagents from the processing chamber 16. In one embodiment, this is accomplished by using a pump 72 and a series of valves 74, 76, 78, 80. The pump 72 and valves 74, 76, 78, 80 are contained in the pressure modifier 44 to direct and control the pressure and vacuum. A pressure sensor 38 senses pressure or vacuum in the processing chamber 16. The pump 72 in the Reagent Module cycles on and off as needed to lift fluids into the processing chamber 16 and to drain fluids to their containers 40. In a first state of operation, when valves V1, V2 (74, 78) are closed and valves V3, V4 (76, 80) are open, the pump 72 acts to create a vacuum in the processing chamber 16. In this manner, liquids are drawn into the chamber. Air from the processing chamber 16 is sent to the filter 50, which is described subsequently. In

a second state of operation, when valves V3, V4 (76, 80) are closed and valves V1, V2 (74, 78) are open, the pump 72 acts to create a pressure in the processing chamber 16. In this manner, liquids are expelled from the chamber, draining into their respective containers. Air is sent to the processing chamber from a vent. This allows for filling and draining the processing chamber while the specimens remain stationary in the processing chamber. This also permits placing the specimens under vacuum or pressure cycles while immersed in solvents or paraffin to permit thorough infiltration. A pressure differential is created between the storage container and the processing chamber using the pump, to move the reagents. Alternatively, the force of gravity may be used to move the reagents or paraffin. Further, as shown in Figure 3, there is a float valve 82 which prevents reagents from the processing chamber 16 to enter the pressure modifier 44 in the event that the fluid level in the processing chamber 16 is too high.

The Reagent Module 14 has electrical cables for the pump 72, pneumatic valves, 74, 76, 78, 80, rotary valve 42 and blower 48. The Reagent Module 14 features a ventilation system 46 that uses activated charcoal filters to collect solvent fumes before they can escape into the atmosphere. The processing and reprocessing system design reduces the production of fumes. The system handles the fumes from these sources with a built-in ventilating system that filters the air through activated charcoal granules. The system consists of a blower 48 and two filter sections: one for solvent fumes 50, the other for formaldehyde fumes 52. The blower 48 draws air through the Reagent Module 14, up through the filters, and out the back of the unit. This filter system allows operation of the system without the need for a fume hood, external ventilating system, or exhaust fan.

The Operating Module 12 further includes a control device 28. The control device, in one embodiment, may be a general purpose computer. This control device 28 automatically controls and sequences the operation of the heaters, motors, pumps and valves, which are controlled via cables. The control device 28 includes, in a preferred embodiment, a processor 54, and in particular, a Hitachi HD-64180 (Z-80) microcontroller. The control device may also include an electro-mechanical timer, an embedded microprocessor circuit, a programmable logic controller, an external computer, or any combination of the above. The control device 28, in one embodiment, contains memory 56 or other computer readable storage medium, including both random access memory (RAM) 58 and read only memory (ROM) 60 in the form of an erasable programmable read only memory (EPROM). The EPROM contains the system operating program and the text and screen formats for the display. Referring to Appendix A and incorporated herein by reference, there is listed the software having a set of instructions for reprocessing of a specimen. The software is written in Z-80 assembly programming language and is executed on the Hitachi HD-64180 (Z-80) microcontroller.

The control device 28 reads the temperature (via a temperature sensor 39), pressure (via a pressure sensor 38) and the processing chamber fluid level (via a fluid level sensor 37) through the Interface Board 62 and controls the heaters and motors through the Power Board 64. The Power Board 64 contains the drivers 65 for the motors 72, heaters 21, valves 34, 42 and the stirrer 36.

The control device 28 further communicates with the Input/Output device 30 or other user interface. The Input/Output device 30 includes a control panel 18 featuring a monitor 66 such as liquid crystal display (LCD) for displaying menus,

instructions and message. The Input/Output device 30 also includes a keypad 68, such as a numeric keypad and an alpha-numeric keyboard or other means for input such as a mouse. The LCD screen assists in programming and operating the system. Through menus, the screen shows status, guides the operator in writing and running reprocessing programs, and serves a variety of maintenance functions. During processing, the monitor 66 shows where specimens are in the cycle, the time in each station, the solution in that station, temperature, and vacuum or pressure. The Input/Output device 30 further includes external ports 70 for connections to external devices such as a printer or a phone line.

The control device 28, in combination with the Input/Output device 30, gives the system its programming flexibility. The operator can program each of the stations (twelve solvent stations and three paraffin stations) for process time, temperature, vacuum or pressure. The monitor 66 displays all parameters to help the operator while writing the program. A variety of menus give the operator the flexibility of performing a variety of maintenance and service procedures. A special help function gives on-screen assistance at any time without affecting the present status. The operator can tailor processes to match tissue requirements for different solutions and soaking times as well as a combination of heat, pressure and vacuum.

**Processing and Reprocessing Cycles**

A processing and reprocessing cycle, in one embodiment, consists of filling the processing chamber with a reagent, processing for a programmable amount of time under conditions of controlled temperature, pressure (or vacuum) and agitation. Then draining the reagent back into its storage container. Examples of cycles of the

specimen reprocessing and processing system are the fill cycle, the drain cycle and the process cycle.

Fill Cycle

As described previously, four pneumatic valves V1, V2, V3, V4 (74, 76, 78, 80) and the pump 72 perform these cycles, all under computer control.

Before the fill cycle, the system checks that the paraffin oven 32, processing chamber 16 and rotary valve block 34 are up to the programmed temperature. The system then vents the processing chamber 16 and calibrates the pressure sensor 38. The solvent rotary valve 42 moves to the proper position for the selected station and the processing chamber rotary valve 34 moves to the closed position for that station. The system then sets the solenoid valves for vacuum and starts the pump 72. This verifies that the processing chamber 16 and pressure modifier 44 do not leak. If the processing chamber 16 cannot maintain vacuum, the solenoid valves cycle five times to clear any contamination from the valve seats. The system makes a second attempt to establish vacuum in the chamber. If the processing chamber 16 still cannot maintain vacuum, the system goes to error standby.

If the system successfully established vacuum, then the system vents the processing chamber 16. The processing chamber rotary valve 34 moves to the open position for the selected station. The system sets the solenoid valves 74, 76, 78, 80 for vacuum and cycles the pump 72 on and off to maintain fill vacuum (4 In. Hg for Stations 1-6, 6 in. Hg for Stations 7-10 and purge stations 15 and 16, 2 in. Hg for paraffin stations 11-13, as shown subsequently in Table 1). The system maintains vacuum until the solution triggers the selected level sensor. The processing chamber rotary valve 34 then closes, and the system vents the processing chamber 16.

Drain Cycle

At the beginning of the drain cycle, the system verifies that the paraffin oven 32, processing chamber 16 and valve block 34 are up to temperature. The system then vents the processing chamber 16 and calibrates the pressure sensor 38 by waiting until there is no change in pressure for 1/4 second. The system then stores the pressure sensor reading as the ambient pressure.

The solvent rotary valve 42 moves to the selected station if the station is a solvent station, then the processing chamber rotary valve 34 moves to the closed position for that station. The system next sets the solenoid valves for pressure 74, 76, 78, 80 and starts the pump 72. Similar to the fill cycle, it does this to verify that the processing chamber 16 and the pressure modifier 44 do not leak. If the processing chamber 16 cannot maintain pressure, the solenoid valves will cycle five times to clear any contamination from the valve seats. The system then makes a second attempt to establish pressure in the processing chamber. If the processing chamber still cannot maintain pressure, the system goes to error standby.

The system then releases pressure. The processing chamber rotary valve 34 moves to the open position for the selected station. The system sets the solenoid valves 74, 76, 78, 80 to pressure and the pump 72 starts cycling on and off to maintain drain pressure (1 psi). The system will maintain drain pressure until the processing chamber 16 can no longer hold pressure, indicating that it is empty (the system senses this by the duration of the pump's running cycle). When the system can no longer hold pressure, it vents the processing chamber 16, then waits five seconds for any remaining fluid to collect in the bottom of the processing chamber 16 and its associated plumbing. The system then sets the solenoid valves 74, 76, 78, 80 to

pressure, and turns the pump 72 on for two seconds to clear the processing chamber 16 and plumbing of any remaining fluid. The system then vents the processing chamber 16 to release any remaining pressure.

Process Cycle

A programming option allows the specimen processing and reprocessing system to alternate pressure and vacuum while processing or reprocessing tissue to enhance the infiltration of the tissue samples. During programming, the operator sets the values: up to seven pounds per square inches of pressure and a vacuum of up to fifteen inches of mercury. Before the system begins the pressure cycle, it vents the pump while maintaining pressure in the processing chamber. The system does this so that the pneumatic pump starts with no load. After the system starts the pump 72, it sets the solenoid valves 74, 76, 78, 80 to pressure. The cycle runs for 3 minutes at each setting, alternating between vacuum and pressure.

**Processing of Specimen**

As one example of the specimen processing and reprocessing system, the reagents are arranged in 15 "stations" (3 paraffin stations and 12 solvent stations).

| Station | Reagent | Concentration | Description |
|---|---|---|---|
| 1 | Formalin | | Fixative |
| 2 | Formalin | | Fixative |
| 3 | Isopropyl Alcohol | 70% | Dehydrant |
| 4 | Isopropyl Alcohol | 95% | Dehydrant |
| 5 | Isopropyl Alcohol | 95% | Dehydrant |
| 6 | Isopropyl Alcohol | 100% | Dehydrant |
| 7 | Isopropyl Alcohol | 100% | Dehydrant |
| 8 | Alcohol/Xylene | 50/50 | Dehydrant |
| 9 | Xylene | | Clearant |
| 10 | Xylene | | Clearant |
| 11 | Paraffin | | |
| 12 | Paraffin | | |
| 13 | Paraffin | | |
| 14 | Xylene | | Purge Clearant |

| | | | |
|---|---|---|---|
| 15 | Isopropyl Alcohol | 100% | Purge Dehydrant |

**Table 1 - Reagent Stations**

Depending on the needs in processing the specimen, any number of stations may be present in the machine. In an alternative embodiment in which the specimen is moved from one container of reagents to the next, there may be 15 such containers, as corresponding to the reagents in Table 1, with as a robotic arm and controls to move the specimen from container to container as necessary.

Referring to Figure 5, there is shown a flow chart of a processing of a specimen. The first step involves fixation of the specimen, as shown at block 84. This typically involves immersing the specimen in Formalin, a fixative. In one embodiment of the invention, the specimen is immersed in, subjected to or exposed to a fixing agent at a station, or a multitude of stations, in a processing machine (*see e.g.*, Stations 1 and 2, as shown in Table 1). However, in processing of the sample, the operator may choose to use both stations, only one station or none of the stations (if the specimen has already been immersed in or exposed to a fixative). The specimen is then dehydrated using a dehydrating agent such as alcohol, as shown at block 86. In one embodiment of the invention, the specimen is dehydrated by immersion in, exposure to or being subjected to a series of alcohol reagents with increasing concentration (*see e.g.*, Stations 3-8, as shown in Table 1). The operator of the processing machine may design a single, or a series, of exposures to alcohol depending on the amount of water contained in the specimen. Thereafter, the specimen is cleared of the dehydrant using a clearing agent, such as xylene (*see e.g.*, Stations 9 and 10, as shown in Table 1), as shown at block 88. Again, depending on processing needs, the specimen may be immersed in, exposed to, or subjected to a

single station or both stations. Thereafter, the specimen is infiltrated with an infiltrating medium such as paraffin (*see e.g.*, Stations 11-13, as shown in Table 1), as shown at block 90.

After the specimen has been processed, the machine should be cleaned in order to minimize contaminants of the reagents upon next use the machine. First, a purge clearant, such as xylene, is used in order to clean the paraffin in the processing chamber and the rotary valve on the processing chamber. Second, a purge dehydrant is used to clean any oily residue, or other contaminants, which may be left in the processing chamber. The purge clearant at station 14 is considered to have more impurities of paraffin and other contaminants than, for example, the clearant at station 9. Further, the purge dehydrant at station 15 is considered to have more impurities of oily residue and other contaminants than, for example, the clearant at station 8.

After the tissue has been processed, it is infiltrated with paraffin, embedded in a paraffin block, and sliced into sections using a microtome. At that point, the operator can determine if the specimen has been processed properly. In one instance, the operator may wish to reprocess the remainder of the sample (*i.e.*, the portion of the specimen which has not been sliced up) until the rehydration of the specimen with an aqueous fluid (to a fixing agent, such as formalin, or to water). If that is the case, the operator indicates, via the control panel 18, that the specimen is to be reprocessed. In addition, if the operator wishes to reprocess a section of the specimen, such as for ploidy analysis, the operator indicates, via the control panel 18, to reprocess the specimen.

**Reprocessing of Specimen**

Referring to Figure 6a, there is shown a flow chart of the automatic reprocessing of the tissue until rehydration of the specimen and then processing of the specimen. The system, in one embodiment, may wait until the operator has signaled to reprocess the specimen, as shown at block 92. The software therefore has an initiating routine, waiting until the operator has initiated reprocessing. To reprocess tissue, the infiltrating medium is first removed. Typically, a specimen is not only infiltrated with a medium, but also embedded or encased in the same medium. For example, a specimen may be infiltrated with paraffin, and for ease of slicing, may also be embedded or enveloped with a paraffin shell. To remove the paraffin shell, the operator may simply slice the shell away from the specimen with a knife. Otherwise, the operator may allow the reprocessing machine to remove the shell of paraffin, as shown at block 94. Operator input, via the control panel 18, indicates whether the paraffin station(s) are to be used. This may optionally be done by running processing cycles with one or more paraffin stations. (*see e.g.*, Stations 11-13, as shown in Table 1). The shell of paraffin is removed from the specimen by raising the temperature of the tissue to the melting point of the paraffin that has infiltrated the tissue, as shown at block 96.

If the paraffin stations have already been run, the valve 34 and processing chamber 16 are contaminated with paraffin; therefore, the purge clearant should be used. As described previously, the purge clearant is typically used in cleaning the valve 34 and processing chamber 16 when processing a sample. Thus, the purge clearant is already contaminated with paraffin and may clean the valve 34 and processing chamber 16. As a general matter (even if the paraffin station(s) have not been run), the order of the clearant stations may optionally be from the most

contaminated (with paraffin) to the cleanest. This is due to the fact that in removing the paraffin, the clearing agent may become contaminated. In order to avoid contamination of the "cleaner" clearants, the purge clearant should be used first. Otherwise, the cleaner clearants (such as Stations 9 or 10) would be contaminated with paraffin if used directly after a paraffin step. If that were the case, upon processing of a sample again, the clearant in station 9 or 10 would have to be replaced due to contamination. Therefore, the specimen is immersed in, subjected to or exposed to a purge clearant first, as shown at block 98. (*see e.g.*, Station 14, as shown in Table 1). Typically, the specimen is immersed or exposed to the purge clearant for about 20 minutes with the agitator 36 mixing.

The next step is the removal of residual infiltrating medium from a specimen with "cleaner" clearing agent(s) (an agent that is miscible with the embedding and dehydrating agent), as shown at block 100. This is done by running processing cycles with one or more clearant stations, depending on the needs of reprocessing. (*See e.g.*, Stations 9 and 10, as shown in Table 1). The clearant typically used to remove the paraffin is Xylene.

Again, the valve 34 and the processing chamber 16 may be contaminated with an oily residue left by the clearant. Therefore, the specimen may optionally be immersed in, subjected to or exposed to a purge dehydrant before other dehydrants, as shown at block 102. (*see e.g.*, Station 15, as shown in Table 1). As described previously, the purge dehydrant is typically used in cleaning the valve 34 and processing chamber 16 when processing a sample. Thus, the purge dehydrant is already contaminated. Otherwise, the cleaner dehydrants (such as stations 3-8) would be contaminated with the oily residue if used directly after a clearant step. If that were

the case, upon processing of a sample again, the dehydrants would have to be replaced due to contamination. Therefore, the specimen may be immersed or exposed to the purge dehydrant for about 20 minutes with the agitator 36 mixing.

The next step is the removal of the residual clearing agent by saturating the specimen with a dehydrating agent, as shown at block 104. This step is performed whether or not the purge dehydrant is used. This is accomplished by running processing cycles with dehydrants (typically alcohol) with successively higher concentrations of water in which the specimen is immersed in, subjected to or exposed to dehydrant(s). One or many of the dehydrant stations may be used, depending on the needs of reprocessing. (*See e.g.*, Stations 3-8, as shown in Table 1).

The next step is the removal of the dehydrating agent with an aqueous fluid, as shown at block 106 by the specimen immersed in, exposed to or subjected to the aqueous fluid. The aqueous fluid can be used for storage (such as using an aqueous fluid comprised of water) or used to complete the fixation process prior to the repeating of the specimen processing (such as using an aqueous fluid comprised of a fixative such as formalin).

Optionally, the program may wait to determine if the operator has indicated to process specimen, as shown at block 108. The operator may indicate to process specimen at the beginning of the reprocessing sequence, in the middle of reprocessing, or after reprocessing has completed. Alternatively, the program may immediately processes the specimen without operator input.

The fixed specimen is then processed similar to the process steps of Figure 5. In particular, the refixed specimen is dehydrated using a dehydrant (such as alcohol), as shown at block 110. The dehydrant in the specimen is then replaced using a

clearing agent (such as xylene), as shown at block 112. The clearing agent is then replaced using an infiltrating medium (such as paraffin), as shown at block 114.

Referring to Figure 6b, there is shown a flow chart of an alternate embodiment of the automatic reprocessing of the tissue and then processing of the specimen. The operator, after slicing of the specimen, may be able to determine which step in the previous processing sequence was faulty. For example, if the clearant in the processing sequence was contaminated, upon processing, the clearant may have failed to clear all of the dehydrant, thus leaving the specimen with residual dehydrant. Based on this observation, the operator may enter in the control panel 18 the step to which reprocessing should be done, as shown at block 115. This entry may be stored in a look-up table 59 in RAM 58, so that upon reprocessing, the software may determine which step to reprocess to. Alternatively, the entry in the look-up table may be the step in the processing sequence which was faulty. In the example given above, the step would be the clearing step. In this manner, the reprocessing program may read the entry in the look-up table 59, and stop the reprocessing either at the faulty step or the step prior to the faulty step.

Similar to Figure 6a, the system waits until the operator has signaled to reprocess the specimen, as shown at block 116. The operator may allow the reprocessing machine to remove the shell of paraffin, as shown at block 118. The shell of paraffin is removed from the specimen by raising the temperature of the tissue to the melting point of the paraffin that has infiltrated the tissue, as shown at block 120. If the paraffin stations have already been run, the order of the clearant stations is from the most contaminated (with paraffin) to the cleanest. Therefore, the purge

clearant is used first, as shown at block 122. (*see e.g.*, Station 14, as shown in Table 1).

The next step is the removal of residual infiltrating medium from the specimen with a clearing agent (an agent that is miscible with the embedding and dehydrating agent), as shown at block 124. Thereafter, the look-up table 59 is examined to determine whether the clearing step is the last or final step in the reprocess, as shown at block 126. If so, the program then determines if the operator has indicated to process the specimen, as shown at block 128. If so, the program goes to the infiltrating step, as shown at block 146. If the clearing step is not the last or final step in the reprocess, the purge dehydrant is used before other dehydrants, as shown at block 130. (*see e.g.*, Station 15, as shown in Table 1).

The next step is the removal of the residual clearing agent by saturating or exposing the specimen with a dehydrating agent, as shown at block 132. This step is performed whether or not the purge dehydrant is used. One or many of the dehydrant stations may be used, depending on the needs of reprocessing. (*See e.g.*, Stations 3-8, as shown in Table 1).

Thereafter, the look-up table 59 is examined to determine whether the dehydrating step is the last or final step in the reprocess, as shown at block 134. If so, the program then determines if the operator has indicated to process the specimen, as shown at block 136. If so, the program goes to the clearing step, as shown at block 144. If the dehydrating step is not the last step in the reprocess, the next step is the removal of the dehydrating agent with an aqueous fluid, as shown at block 138. Optionally, the program then waits to determine if the operator has indicated to process specimen, as shown at block 140; otherwise, processing begins without

operator input. The operator may indicate to process specimen at the beginning of the reprocessing sequence, in the middle of reprocessing, or after reprocessing has completed. The fixed specimen is then processed similar to the process steps of Figure 5. In particular, the refixed specimen is dehydrated using a dehydrant (such as alcohol), as shown at block 142. The dehydrant in the specimen is then replaced using a clearing agent (such as xylene), as shown at block 144. The clearing agent is then replaced using an infiltrating medium (such as paraffin), as shown at block 146.

From the foregoing detailed description, it will be appreciated that numerous changes and modifications can be made to the hardware and software aspects of the invention without departure from the true spirit and scope of the invention. For example, the present invention is not dependent on any specific type of computer architecture or type of protocol. This true spirit and scope of the invention is defined by the appended claims, to be interpreted in light of the foregoing specification.

Appendix A

```
******************************************************************
*
*       REVERSE RUN
*
******************************************************************

REVRUN:         CALL    DISREV

REVR2: CALL     GETKEY
        CP      KBF1            ;START
        JP      Z,RUNREV
        CP      KBF2            ;EDIT
        JP      Z,EDITREV
        CP      KBF3            ;REPROCESSING PROGRAM
        JP      Z,REPCHG
        CP      KBF4            ;CANCEL
        JP      Z,MAINTN
        JR      REVR2

        RET

******************************************************************
*
*       DISPLAY PROGRAM - REVERSE
*
******************************************************************

DISREV:         LD      A,(REVSTEP)
        PUSH    AF
        LD      A,(STANUB)
        PUSH    AF

        LD      A,81
        LD      (SCRNUB),A
        CALL    DISPSR

        LD      A,2
        LD      (DISROW),A
        LD      A,3
        LD      (DISCOL),A
        CALL    PTSET                   ;POINT AT PROGRAM

        XOR     A
DISRE1:         INC     A
        PUSH    AF

        LD      (REVSTEP),A
        CALL    SOREV
        CALL    GETRRS
        CALL    BLKDAT                  ;BLANK DSPDAT
        LD      HL.DSPDAT               ;BUFFER AREA
        LD      (HL),00
        INC     HL
        LD      A.(STANUB)
        CALL    BINASC
```

```
        INC     HL
        CALL    LINEDT                  ;LINE AT A TIME

        LD      A,(DISROW)
        INC     A
        LD      (DISROW),A
        LD      A,2
        LD      (DISCOL),A
        CALL    PTSET                   ;POINT AT PROGRAM

        LD      HL,DSPDAT
        CALL    MESOUT

        POP     AF
        CP      15
        JR      NZ,DISRE1

        XOR     A
        LD      (EDVAR),A               ;ENABLE PROCESS TIME ROUTINE
        CALL    REVTPT                  ;DISPLAY TOTAL PROCESS TIME
        CALL    DISSS                   ;DISPLAY STIR SPEED
        CALL    DISLS                   ;DISPLAY LEVEL SENSOR
        CALL    DSPRPN                  ;DISPLAY REPROCESSING NAME

        POP     AF
        LD      (STANUB),A
        POP     AF
        LD      (REVSTEP),A
        RET

*****************************************************************
*
*       REVERSE RUN - RUN
*
*****************************************************************

RUNREV: XOR     A
        LD      (BEEPFLG),A             ;TURN BEEPER OFF

        IN0     A,(PORT1C)
        RES     5,A                     ;DISABLE ALARM RELAY
        OUT0    (PORT1C),A

        CALL    ALLOFF
        CALL    ERRSAV

        LD      A,(FLAG3)               ;CHECK IF POT FULL
        BIT     6,A                     ;
        JP      NZ,CUSERV               ;IF SO GO TO USER SERVICE

        LD      A,84                    ;SCREEN 1.2.4
        LD      (SCRNUB),A              ;POWER FAIL REENTRY
        CALL    FUNCT

        LD      A,1
        LD      (REVSTEP),A
```

```
        CALL  ERRCLR       ;CLEAR ALL ERRORS.

        LD    HL,FPCDAT    ;SET HOLD TIME TO CURRENT TIME
        LD    DE,HDATE
        LD    A,4
        CALL  COPYN

        LD    HL,FPCTIM
        LD    DE,HTIME
        LD    A,4
        CALL  COPYN

        CALL  ACTREV       ;GET FIRST ACTIVE STATION.
        OR    A            ;IS ANY ACTIVE?
        JP    NZ,RRUN2     ;EXIT IF NO STATIONS ACTIVE

        LD    A,(REPNUB)
        OR    A
        JP    NZ,RRUN14
        JP    MAINTN

RRUN2:CALL  ACTREV       ;GET NEXT ACTIVE STATION.

        LD    A,(REVSTEP)
        LD    (LASTST),A

        CALL  STTITL       ;INITIALIZE STATS.
        CALL  ITLVRP       ;INIT LEVEL STAT

        CALL  SOREV
        LD    A,(STANUB)
        CALL  GETRRS
        CALL  SETSTA
        CALL  FINISH

        XOR   A
        LD    (DSPRMS),A

        CALL  SPTIME

RRUN3:LD    A,1          ;RUN STATE FILL
        LD    (BSTATE),A

        CALL  DISREV

        LD    A,(REVSTEP)
        CALL  PGMPTR

        LD    A,(FLAG3)    ;THIS IS FOR REENTRY
        BIT   6,A
        JR    NZ,RRUN6     ;IF POT FULL DO NOT RESTART STATION

        LD    A,(FLAG1)
        SET   0,A          ;INFILTRATION FLAG
        LD    (FLAG1),A

        CALL  LIDCK
```

```
        CALL  GETRRS          ;GET STATION DATA
        CALL  SETSTA          ;SET STATION PARAMETERS.
        CALL  FILPOT          ;FILL TISSUE POT

        LD    A,(STIRSP)
        LD    (STIRS1),A
        CALL  SPEED

        LD    A,(STANUB)      ;CLEAR PURGE FLAG IF PURGE STATIONS RUN
        CP    14
        JR    NZ,RRUN4
        LD    A,(PRGFLG)
        RES   0,A
        LD    (PRGFLG),A
        JR    RRUN5

RRUN4   CP    15
        JR    NZ,RRUN5
        LD    A,(PRGFLG)
        RES   1,A
        LD    (PRGFLG),A

RRUN5   LD    A,6             ;INIT V/P FLAGS.
        LD    (VPTIM),A

        CALL  STATIT          ;INITIALIZE POT STAT.

RRUN6:  CALL  LIDCK

        LD    A,2             ;RUN STATE PROCESSING
        LD    (BSTATE),A

        CALL  DISREV

        XOR   A
        LD    (DSPRMS),A      ;BLANK RUN MESSAGE.

        CALL  ERRSAV

        XOR   A
        LD    (ESTATE),A      ;RESET ERROR STATE

        LD    A,(STANUB)
        CALL  GETRRS
        CALL  SETSTA
        LD    A,(REVSTEP)
        CALL  PGMPTR

        CALL  CLRRX           ;CLEAR ALL KEYS ENTERED

        CALL  STOPST          ;ENABLE STOP KEY

        LD    A,(FLAG4)
        RES   3,A             ;ENABLE VALVES
        LD    (FLAG4),A
```

```
        LD      A,(DSPFLG)
        RES     0,A                     ;DISPLAY ALL FIRST TIME
        LD      (DSPFLG),A

***************************************************************

RRUN7:CALL   INPUT                   ;TEST FOR STOP

        CALL    STIRON
        CALL    POTON
        CALL    VPRUN

        CALL    DSPRUN
        CALL    STATCHK
        CALL    LIDCK

        LD      A,(CLKFLG)
        BIT     0,A                     ;TIMED OUT YET?
        JP      Z,RRUN7

***************************************************************

        CALL    ALLOFF
        CALL    STOPUS                  ;DISABLE STOP KEY

        LD      A,(STANUB)
        CALL    ERRSAV
        CALL    SVSTAT

        LD      A,(REVSTEP)
        INC     A
        LD      (REVSTEP),A

        CALL    ACTREV                  ;GET NEXT ACTIVE STATION.
        OR      A
        JR      Z,RRUN10

RRUN8:LD     A,(LASTST)              ;RECALL LAST
        LD      (REVSTEP),A

        CALL    DISREV

        CALL    SOREV
        LD      A,(STANUB)
        CALL    GETRRS

        LD      A,(REVSTEP)
        CALL    PGMPTR                  ;POINT TO STATION.

        LD      A,3
        LD      (BSTATE),A              ;RUN STATE DRAIN

        CALL    DRAIN

        LD      A,1                     ;MSG1 LOC.
        LD      (DSPRMS),A
        CALL    RUNMSG                  ;DISPLAY DRAIN MESS
```

```
RRUN9 CALL  INPUT            ;CHECK FOR STOP

      LD    A,(CLKFLG)
      BIT   1,A
      JR    Z,RRUN9

      CALL  ERRSAV

      LD    A,(REVSTEP)
      INC   A
      LD    (REVSTEP),A

      JP    RRUN2

RRUN10:     CALL  ALLOFF     ;PROCESS COMPLETE

      CALL  DISREV

      LD    A,(REPNUB)
      OR    A
      JR    NZ,RRUN13

      LD    A,(LASTST)       ;RECALL LAST
      LD    (REVSTEP),A
      CALL  SOREV
      LD    A,(STANUB)
      CALL  GETRRS
      CALL  SETSTA

      LD    A,8              ;SCREEN 1.2.4
      LD    (SCRNUB),A       ;POWER FAIL REENTRY
      CALL  FUNCT
      LD    A,(REVSTEP)
      CALL  PGMPTR

      CALL  STIRON

      LD    A,5
      LD    (BSTATE),A       ;RUN STATE PROCESS COMPLETE

      LD    A,(REVSTEP)
      CALL  PGMPTR           ;POINT TO STATION.

      LD    A,6              ;PROCESSING COMPLETE.
      LD    (DSPRMS),A
      CALL  RUNMSG

RRUN11      CALL  GETKEY
      CP    KBF1             ;DRAIN
      JR    Z,RRUN12
      JR    RRUN11

RRUN12:     CALL  ALLOFF

      LD    A,(STANUB)
      CALL  SVSTAT
```

```
        CALL    ERRSAV

        CALL    DRAIN

        CALL    ERRSAV              ;SAVE ERRORS
        XOR     A
        LD      (BSTATE),A          ;DEACTIVATE RUN

        LD      A,(PRGFLG)          ;CHECK IF PARAFFINS
        AND     03H                 ;HAVE RUN.
        LD      (PRGFLG),A

        LD      A,(FLAG2)
        RES     7,A
        LD      (FLAG2),A           ;CLEAR LID OPEN FLAG

        JP      MAINTN

RRUN13:         LD      A,(LASTST)  ;DRAIN AND JUMP TO PROCESSING PROGRAM
        LD      (REVSTEP),A         ;RECALL LAST

        CALL    DISREV

        CALL    SOREV
        LD      A,(STANUB)
        CALL    GETRRS

        LD      A,(REVSTEP)
        CALL    PGMPTR              ;POINT TO STATION.

        LD      A,6
        LD      (BSTATE),A          ;RUN STATE DRAIN

        CALL    DRAIN

RRUN14:         CALL    ERRSAV      ;SAVE ERRORS
        XOR     A
        LD      (BSTATE),A          ;DEACTIVATE RUN

        LD      A,(PRGFLG)          ;CHECK IF PARAFFINS
        AND     03H                 ;HAVE RUN.
        LD      (PRGFLG),A

        LD      A,(FLAG2)
        RES     7,A
        LD      (FLAG2),A           ;CLEAR LID OPEN FLAG

        LD      A,(REPNUB)
        LD      (PGMNUB),A
        CALL    GETPGM

        CALL    FINISH              ;COMPUTE IMEDIATE FINISH TIME
        XOR     A
        LD      (STANUB),A
        JP      RUN1

**************************************************************
```

```
*
*       GETS NEXT ACTIVE STATION AND PUTS IT IN REVSTEP. 00H IF NOT. - REV
*
****************************************************************

ACTREV:         LD      A,(REVSTEP)
        PUSH    AF

ACTR1:  CALL    SOREV
        LD      A,(STANUB)

        CALL    STONCK
        OR      A                       ;IS STATION OFF?
        JR      NZ,ACTR3

        LD      A,(REVSTEP)
        CP      15                      ;IS NUMBER BEYOND 15?
        JP      P,ACTR2
        INC     A                       ;ADVANCE STATION.
        LD      (REVSTEP),A
        JR      ACTR1

ACTR2:  POP     AF
        LD      (REVSTEP),A
        XOR     A
        RET

ACTR3:  POP     AF
        RET

****************************************************************
*
*       OUTPUT TOTAL PROCESS TIME - REV
*
****************************************************************

REVTPT:         PUSH    AF
        PUSH    DE
        PUSH    HL

        LD      A,(EDVAR)
        OR      A
        JR      NZ,REVT2

        LD      HL,FDATE
        LD      DE,SDATE
        LD      A.6
        CALL    COPYN

        LD      DE,CTIM1                ;ZERO ACCUM
        LD      HL,ZERO
        CALL    KOPY

        XOR     A
        LD      (STAON1),A              ;INIT STATION BITS.
        LD      (STAON2),A
```

```
         LD     A,(STANUB)
         CALL   SAVRRS          ;SAVE STADAT
         PUSH   AF

         LD     A,15
         LD     (STANUB),A

REVT1:   CALL   GETRRS          ;GET NEXT STATION
         LD     BC,TIME1
         LD     HL,TMPR1
         CALL   CFLPT           ;CONVERT TO FLOATING

         CALL   STAON           ;CHECK IF STATION ON.
         LD     IY,CTIM1
         LD     BC,TMPR1
         LD     HL,CTIM1
         CALL   TIMADD          ;ACCUM TIME

         LD     A,(STANUB)
         DEC    A
         LD     (STANUB),A
         JR     NZ,REVT1        ;TEST IF 15 STATION

         LD     HL,PTIME
         LD     BC,CTIM1        ;SAVE TOTAL PROCESS TIME
         LD     A,20H
         CALL   CASCI

         LD     A,18            ;SET POINTER TO STATION
         LD     (DISROW),A
         LD     A,10
         LD     (DISCOL),A
         CALL   PTSET
         CALL   BLKDAT

         LD     HL,DSPDAT
         LD     DE,PTIME        ;OUTPUT PROCESS TIME
         CALL   SFTIME
         LD     (HL),1AH

         LD     HL,DSPDAT
         CALL   MESOUT

         POP    AF
         LD     (STANUB),A
         CALL   GETRRS          ;RESTORE STADAT

REVT2    POP    HL
         POP    DE
         POP    AF
         RET

***************************************************************
*
*        REVERSE RUN - EDIT
*
***************************************************************
```

```
EDITREV: LD   A,82
         LD   (SCRNUB),A
         CALL FUNCT              ;DISPLAY SCREEN

         LD   A,(FLAG1)
         RES  4,A
         LD   (FLAG1),A          ;CLEAR EDITOR RANGE ERROR

         LD   A,1
         LD   (REVSTEP),A
         XOR  A
         LD   (EDVAR),A

RRE1:    LD   A,(REVSTEP)
         CALL SOREV
         CALL GETRRS

         LD   A,(FLAG1)
         SET  7,A
         LD   (FLAG1),A          ;SET REV VIDEO FLAG

         LD   A,(REVSTEP)
         CALL EDLINE

RRE2:    CALL GETKEY
         CP   KBUP               ;REV FIELD
         JP   Z,RRFREV
         CP   KBDN               ;ADV FIELD
         JP   Z,RRFFWD
         CP   KBF1               ;F1 DONE
         JP   Z,REVRUN
         CP   KBF2               ;F2 SOLUTION EDIT
         JP   Z,REVSOL
         CP   KBF3               ;F3 CHANGE STIR SPEED
         JP   Z,REVSPD
         CP   KBF4               ;F4 CHANGE LEVEL SENSOR
         JP   Z,REVLEV
         CP   0DH                ;ENTER
         JP   Z,RRDAT
         CP   KBCL               ;CLEAR ON/OFF
         JR   Z,RONOFF
         AND  07FH
         SUB  030H
         CP   0AH                ;NUMBER
         JP   P,RRE2
         JP   REVNUB

RONOFF        CALL ONOFF
         CALL GETBAC
         CALL SAVRRS
         LD   A,(REVSTEP)
         CALL EDLINE
         JR   RRE2

***************************************************************
*
```

```
*       CHANGE STIR SPEED
*
*************************************************************

REVSPD:         LD      A,(STIRSP)
        INC     A
        CP      11
        JP      M,REVSPD1
        LD      A,0

REVSPD1         LD      (STIRSP),A
        CALL    DISSS
        JP      RRE2

*************************************************************
*
*       CHANGE LEVEL SENSOR
*
*************************************************************

REVLEV:         LD      A,(LEVFLG)
        XOR     002H                    ;TOGGLE
        LD      (LEVFLG),A
        CALL    DISLS
        JP      RRE2

*************************************************************
*
*       CHANGE REPROCESSING PROGRAM
*
*************************************************************

REPCHG:         LD      A,(REPNUB)
        INC     A
        CP      12
        JP      M,REPCHG1
        LD      A,0

REPCHG1         LD      (REPNUB),A
        CALL    DSPRPN
        JP      REVR2

*************************************************************
*
*       INPUT NUMBER INTO VARIABLE POINTED
*       TO BY REVSTEP AND EDVAR
*
*************************************************************

REVNUB:         PUSH    AF
        CALL    PUTNUB

        LD      A.20H
        CALL    FILVAR                  ;FILL VARIABLE WITH BLANKS

        POP     AF
        ADD     A.30H                   ;CONVERT TO ASCII.
```

```
        PUSH  HL
        POP   IY
        LD    (IY+5),A          ;LOAD FIRST CHAR.

        LD    A,C
        CP    5
        CALL  Z,EDDP            ;CHECK IF TIME VARIABLE.
        CP    1
        CALL  Z,EDDP            ;CHECK IF TIME VARIABLE.

        LD    A,(REVSTEP)
        CALL  EDLINE            ;OUTPUT VARIABLE.

REDN1:  CALL  GETKEY
        CP    KBF1              ;CANCEL
        JP    Z,RRE1
        CP    0DH               ;ENTER
        JP    Z,RRDAT
        CP    KBCL              ;CLEAR
        JR    Z,AONOFF
        AND   07FH
        SUB   030H
        CP    0AH               ;NUMBER
        JP    P,REDN1
        DEC   B
        JR    Z,REDN2
        ADD   A,30H
        CALL  SHFTIN            ;SHIFT IN NEW NUMBER.
        LD    A,C
        CP    5
        CALL  Z,EDDP            ;CHECK IF TIME VARIABLE.
        CP    1
        CALL  Z,EDDP            ;CHECK IF TIME VARIABLE.

        LD    A,(REVSTEP)
        CALL  EDLINE            ;OUTPUT VARIABLE.
        JR    REDN1

REDN2:  INC   B
        JR    REDN1

AONOFF        CALL  ONOFF
        CALL  GETBAC
        CALL  SAVRRS
        LD    A,(REVSTEP)
        CALL  EDLINE
        JR    REDN1

*****************************************************************
*
*       SAVES THE FIELD
*
*****************************************************************

RRDAT:  CALL  RNGCHK
        CP    0AAH              ;TEST IF IN RANGE.
        JP    NZ,RRD1
```

```
        CALL  GETRRS
        LD    A,(FLAG1)
        SET   4,A
        LD    (FLAG1),A          ;SET EDITOR RANGE ERROR
        JP    RRE1

RRD1    CALL  SAVRRS
        LD    A,(REVSTEP)
        CALL  OUTLIN
        LD    A,(FLAG1)
        RES   4,A
        LD    (FLAG1),A          ;CLEAR EDITOR RANGE ERROR
        JP    RRFFWD

*************************************************************
*
*       REV FIELD
*
*************************************************************

RRFREV:       LD      A,(FLAG1)
        RES   7,A
        LD    (FLAG1),A          ;CLEAR REV VIDEO FLAG

        BIT   4,A                ;RETURN IF EDITOR RANGE ERROR
        JP    NZ,RRE1

        LD    A,(REVSTEP)
        CALL  EDLINE
        CALL  REVTPT             ;DISPLAY TOTAL PROGRAM TIME

        LD    A,(EDVAR)
        OR    A                  ;FIRST VARIABLE IN STA?
        JR    Z,RRF1
        DEC   A
        LD    (EDVAR),A
        JP    RRE1

RRF1:   CALL  SAVRRS
        LD    A,(REVSTEP)
        CALL  OUTLIN
        CP    1                  ;FIRST STATION?
        JR    Z,RRF2

        DEC   A                  ;ACCESS PREVIOUS LINE
        LD    (REVSTEP),A
RRF4:   CALL  OFFRCK
        OR    A
        JR    NZ,RRF3
        XOR   A
        LD    (EDVAR),A
        JP    RRE1

RRF2:   LD    A,15
        LD    (REVSTEP),A
        JR    RRF4
```

```
RRF3:   LD      A,3                     ;RAP AROUND
        LD      (EDVAR),A
        JP      RRE1

****************************************************************
*
*       ADVANCE FIELD
*
****************************************************************

RRFWD:          LD      A,(FLAG1)
        RES     7,A
        LD      (FLAG1),A               ;CLEAR REV VIDEO FLAG

        BIT     4,A                     ;RETURN IF EDITOR RANGE ERROR
        JP      NZ,RRE1

        LD      A,(REVSTEP)
        CALL    EDLINE
        CALL    REVTPT                  ;DISPLAY TOTAL PROGRAM TIME

RAF0    LD      A,(EDVAR)
        CP      3                       ;FIRST VARIABLE IN STA?
        JR      Z,RAF1
        CALL    OFFRCK
        OR      A
        JR      Z,RAF1

        LD      A,(EDVAR)
        INC     A
        LD      (EDVAR),A
        JP      RRE1

RAF1:   CALL    SAVRRS
        LD      A,(REVSTEP)
        CALL    OUTLIN
        CP      15                      ;FIRST STATION?
        JR      Z,RAF2
        INC     A                       ;ACCESS PREVIOUS LINE
        LD      (REVSTEP),A
        JR      RAF3
RAF2:   LD      A,1
        LD      (REVSTEP),A

RAF3:   XOR     A                       ;RAP AROUND
        LD      (EDVAR),A
        CALL    SOREV
        CALL    GETRRS
        JP      RRE1

OFFRCK: PUSH DE

        CALL    SOREV
        CALL    GETRRS
        LD      DE,TIME1
        CALL    CKZERO
```

```
        POP     DE
        RET

*****************************************************************
*
*       LOADS STATION DATA IN STANUB TO STADAT - REVERSE
*
*****************************************************************

GETRRS: PUSH AF
        PUSH    BC
        PUSH    HL
        PUSH    DE

        LD      A,(STANUB)
        CP      16
        JP      P,GSTR1
        LD      HL,STARR
        DEC     A
        CALL    GETPT
        LD      DE,STADAT
        LD      BC,31
        LDIR
        LD      HL,STADAT
        LD      DE,STADAT1
        LD      BC,31
        LDIR

GSTR1:  POP     DE
        POP     HL
        POP     BC
        POP     AF
        RET

*****************************************************************
*
*       SAVES STATION DATA IN STADAT TO STATION IN STANUB - REVERSE
*
*****************************************************************

SAVRRS: PUSH AF
        PUSH    BC
        PUSH    HL
        PUSH    DE

        LD      A,(STANUB)
        CP      16
        JP      P,SSTR1
        LD      HL,STARR
        DEC     A
        CALL    GETPT
        PUSH    HL
        POP     DE
        LD      HL,STADAT
        LD      BC,31
        LDIR
        LD      HL,STADAT
```

```
        LD    DE,STADAT1
        LD    BC,31
        LDIR

SSTR1:  POP   DE
        POP   HL
        POP   BC
        POP   AF
        RET

*****************************************************************
*
*       EDIT SOLUTION LIST - REVERSE
*
*****************************************************************

REVSOL:       LD      A,83
        LD    (SCRNUB),A
        CALL  DISPSR

        CALL  ORSOL

REVSL1:       LD      A,(FLAG1)
        SET   7,A                     ;REV VIDEO
        LD    (FLAG1),A

        LD    A,(REVSTEP)
        CALL  OUTSOL

REVSL2:       CALL    GETKEY
        CP    030H                    ;SOLUTION LIST
        JP    Z,RSOLST
        CP    KBF1                    ;DONE
        JP    Z,REVRUN
        CP    KBCL                    ;CLEAR
        JP    Z,CLRSOL
        CP    KBDN                    ;FIELD ADV
        JP    Z,RSADV
        CP    KBUP                    ;FIELD REV
        JP    Z,RSREV
        CP    0DH                     ;ENTER
        JP    Z,REVSL4
        AND   07FH
        SUB   030H
        CP    0AH
        JP    M,ENRSOL
        JR    REVSL2

RSOLST:       CALL    SOLIST
        JR    REVSOL

REVSL4:       CALL    SAVRRS
        JR    REVSL1

CLRSOL: LD    A,0
        LD    (SOLUT1),A
        LD    HL,PERCT1
```

```
        LD      A,20H
        CALL    FILVAR
        CALL    SAVRRS
        JR      REVSL1

ENRSOL:         CALL    REVIN
        CALL    SAVRRS
        JR      REVSL1

*******************************************************************
*
*       ADVANCE TO NEXT STATION - REVERSE
*
*******************************************************************

RSADV:  LD      A,(FLAG1)
        RES     7,A                     ;NO REV VIDEO
        LD      (FLAG1),A

        LD      A,(REVSTEP)
        CALL    OUTSOL

        INC     A
        CP      16
        JR      NZ,RSA1
        LD      A,1
RSA1:   LD      (REVSTEP),A
        CALL    SOREV
        CALL    GETRRS
        JP      REVSL1

*******************************************************************
*
*       REVIEW PREVIOUS STATION - REVERSE
*
*******************************************************************

RSREV:  LD      A,(FLAG1)
        RES     7,A                     ;NO REV VIDEO
        LD      (FLAG1),A

        LD      A,(REVSTEP)
        CALL    OUTSOL

        DEC     A
        JR      NZ,RSR1
        LD      A,15
RSR1:   LD      (REVSTEP),A
        CALL    SOREV
        CALL    GETRRS
        JP      REVSL1

*******************************************************************
*
*       OUTPUT 15 STATIONS WITH SOLUTIONS. - REVERSE
*
*******************************************************************
```

```
ORSOL:PUSH   HL
      PUSH   BC

      LD     A,1
      LD     (REVSTEP),A
      CALL   SOREV
      CALL   GETRRS

      LD     A,(FLAG1)
      RES    7,A                 ;NO REV VIDEO
      LD     (FLAG1),A

RSOT1: LD    A,(REVSTEP)
      CALL   OUTSOL

      INC    A
      CP     16
      JR     Z,RSOT2
      LD     (REVSTEP),A
      CALL   SOREV
      CALL   GETRRS
      JR     RSOT1

RSOT2 LD     A,1
      LD     (REVSTEP),A
      CALL   SOREV
      CALL   GETRRS

      POP    BC
      POP    HL
      RET

*******************************************************************
*
*     STATION LIST - REVERSE
*
*******************************************************************

STARR:DW     RR1
      DW     RR2
      DW     RR3
      DW     RR4
      DW     RR5
      DW     RR6
      DW     RR7
      DW     RR8
      DW     RR9
      DW     RR10
      DW     RR11
      DW     RR12
      DW     RR13
      DW     RR14
      DW     RR15

*******************************************************************
*
```

```
*       STATION ORDER LIST - REVERSE
*       GET STANUB FROM REVSTEP
*
*****************************************************************

SOREV:PUSH    AF
      PUSH    BC
      PUSH    HL

      LD      HL,RSTALST
      LD      A,(REVSTEP)
      DEC     A
      LD      C,A
      LD      B,0
      ADD     HL,BC
      LD      A,(HL)
      LD      (STANUB),A

      POP     HL
      POP     BC
      POP     AF
      RET

RSTALST       DB     13
      DB      12
      DB      11
      DB      14
      DB      10
      DB      9
      DB      15
      DB      8
      DB      7
      DB      6
      DB      5
      DB      4
      DB      3
      DB      2
      DB      1
```

## CLAIMS

We claim:

1. An apparatus for automatically reprocessing a specimen from an infiltrating medium to an aqueous fluid comprising in combination:

a processing chamber for holding a specimen;

means for regulating flow of fluid to the processing chamber;

at least one container of a clearant agent, at least one container of a dehydrant agent and at least one container of an aqueous fluid, the containers of clearant, dehydrant and aqueous fluid being connected to the processing chamber via means for regulating flow of fluid to the processing chamber; and

a control device having a processor and a memory device, the processor controlling the means for regulating flow of fluid in order to automatically and sequentially connect the processing chamber with the container of clearant agent, the container of dehydrant agent and the container of aqueous solution in order to reprocess the specimen.

2. The apparatus of claim 1 wherein means for regulating flow of fluid includes a rotary valve and wherein the processor selects the containers of clearant, dehydrant or aqueous fluid by setting the rotary valve.

3. The apparatus of claim 1 further comprising:

at least one container of an infiltrating medium being connected to the processing chamber by a second valve and wherein the processor controls the second

valve.

4. The apparatus of claim 3 wherein the processor further controls the means for regulating flow of fluid and the second valve in order to automatically and sequentially, after the connection to the container of aqueous fluid, connect the processing chamber with the container of dehydrant agent, the container of clearant and the container of infiltrating medium in order to process the specimen.

5. The apparatus of claim 1 further comprising a container of purge dehydrant being connected to the processing chamber by the means for regulating flow of fluid, the processor controlling the means for regulating flow of fluid in order to automatically and sequentially connect the processing chamber with the container of clearant agent, the container of purge dehydrant, the container of dehydrant agent and the container of aqueous solution in order to reprocess the specimen.

6. The apparatus of claim 5 further comprising a container of purge clearant being connected to the processing chamber by the means for regulating flow of fluid, the processor controlling the means for regulating flow of fluid in order to automatically and sequentially connect the processing chamber with the container of purge clearant, the container of clearant agent, the container of purge dehydrant, the container of dehydrant agent and the container of aqueous solution in order to reprocess the specimen.

7. A computer readable storage medium containing a set of instructions

for a general purpose computer having a user interface comprising means for input, an output driver for connections to at least one valve, the valve being connected to at least one container of a clearant agent, at least one container of a dehydrant agent and at least one container of an aqueous fluid, the set of instructions comprising:

an initiating routine operatively associated with said user interface for permitting a user to initiate reprocessing via the means for input, said means for input being associated with a reprocessing program accessible to said computer;

a run routine for implementing said reprocessing program selected by the user, the reprocessing program controlling the output drive to the valve in order to automatically and sequentially connect the valve to the container of clearant agent, the container of dehydrant agent and the container of aqueous solution for reprocessing of a specimen.

8. The computer readable storage medium of claim 7 wherein the valve is connected to a container of purge dehydrant and wherein

the reprocessing program automatically and sequentially connects the valve to the container of purge dehydrant after connection of the valve to the container of clearant agent.

9. The computer readable storage medium of claim 8 wherein the valve is connected to a container of purge clearant and wherein

the reprocessing program automatically and sequentially connects the valve to the container of purge clearant before connection of the valve to the container of clearant agent.

10. Method for automatically reprocessing a specimen using a specimen reprocessing machine having processor for controlling the exposure of the specimen to a clearing agent, a dehydrating agent and an aqueous fluid, the method comprising the steps of:

providing the specimen which is infiltrated with an infiltrating medium;

indicating to the specimen reprocessing machine that the specimen is to be reprocessed;

exposing the specimen to a clearing agent via the processor to remove the infiltrating medium from the specimen; thereafter

exposing the specimen to a dehydrating agent via the processor to remove the clearing agent; and thereafter

exposing the specimen to an aqueous fluid via the processor to remove the dehydrating agent from the specimen.

11. The method of claim 10 wherein the processor further controls the exposure of the specimen to an infiltrating medium and further comprising the steps of:

exposing the specimen to a dehydrating agent via the processor after exposing the specimen to an aqueous fluid;

exposing the specimen to a clearing agent to remove the dehydrating agent; and

exposing the specimen to an infiltrating medium to replace the clearing agent.

12. The method of claim 10 wherein the clearing agent is xylene.

13. The method of claim 10 wherein the dehydrating agent is alcohol.

14. The method of claim 10 wherein the aqueous fluid is formalin.

15. A computer-readable storage medium containing a set of instructions for a general purpose computer, said set of instructions implementing the procedure shown in Figure 6a.

16. Method for reprocessing a specimen which is infiltrated with an infiltrating medium using a specimen reprocessing system having a processing chamber, the method comprising the steps of:

subjecting the specimen to at least one exposure to a clearant;

subjecting the tissue sample to at least one exposure to a purge dehydrant after the exposure to the clearant, the purge dehydrant being contaminated with clearant and being used to clean the processing chamber of the clearant;

subjecting the specimen to at least one exposure to a dehydrant after the exposure to the purge dehydrant; and

subjecting the specimen to an aqueous fluid.

17. The method of claim 16 further comprising the steps of:

subjecting the specimen to at least one exposure to paraffin; and thereafter

subjecting the specimen to at least one exposure to a purge clearant after the

exposure to the bath of paraffin, the purge clearant being contaminated with clearant and being used to clean the processing chamber of paraffin.

18. The method of claim 17 further comprising the steps of:

subjecting the specimen to the dehydrant after subjecting the specimen to an aqueous fluid;

subjecting the specimen to the clearant to remove the dehydrant; and

subjecting the specimen to an infiltrating medium to replace the clearant.

19. Method for reprocessing a specimen which is infiltrated with an infiltrating medium using a specimen reprocessing system having a processing chamber, the method comprising the steps of:

inputting a final step in reprocessing the specimen;

subjecting the specimen to at least one exposure to a clearant;

determining whether the step of subjecting the specimen to at least one exposure of clearant is the final step in reprocessing the specimen;

subjecting the specimen to at least one exposure to a dehydrant if the step of subjecting the specimen to at least one exposure of clearant is not the final step in reprocessing the specimen;

determining whether the step of subjecting the specimen to at least one exposure of dehydrant is the final step in reprocessing the specimen; and

subjecting the specimen to an aqueous fluid if the step of subjecting the specimen to at least one exposure of dehydrant is not the final step in reprocessing the specimen.

20. The method of claim 19 further comprising the step of

subjecting the specimen to an infiltrating medium to replace the clearant, after the step of determining whether the step of subjecting the specimen to at least one exposure of clearant is the final step, if the step of subjecting the specimen to at least one exposure of clearant is the final step in reprocessing the specimen.

21. The method of claim 19 further comprising the steps of:

subjecting the specimen to the clearant to remove the dehydrant, after the step of determining whether the step of subjecting the specimen to at least one exposure of dehydrant is the final step, if the step of subjecting the specimen to at least one exposure of dehydrant is the final step in reprocessing the specimen; and

subjecting the specimen to an infiltrating medium to replace the clearant.

22. The method of claim 19 further comprising the steps of:

subjecting the specimen to the dehydrant after subjecting the specimen to an aqueous fluid;

subjecting the specimen to the clearant to remove the dehydrant; and

subjecting the specimen to an infiltrating medium to replace the clearant.

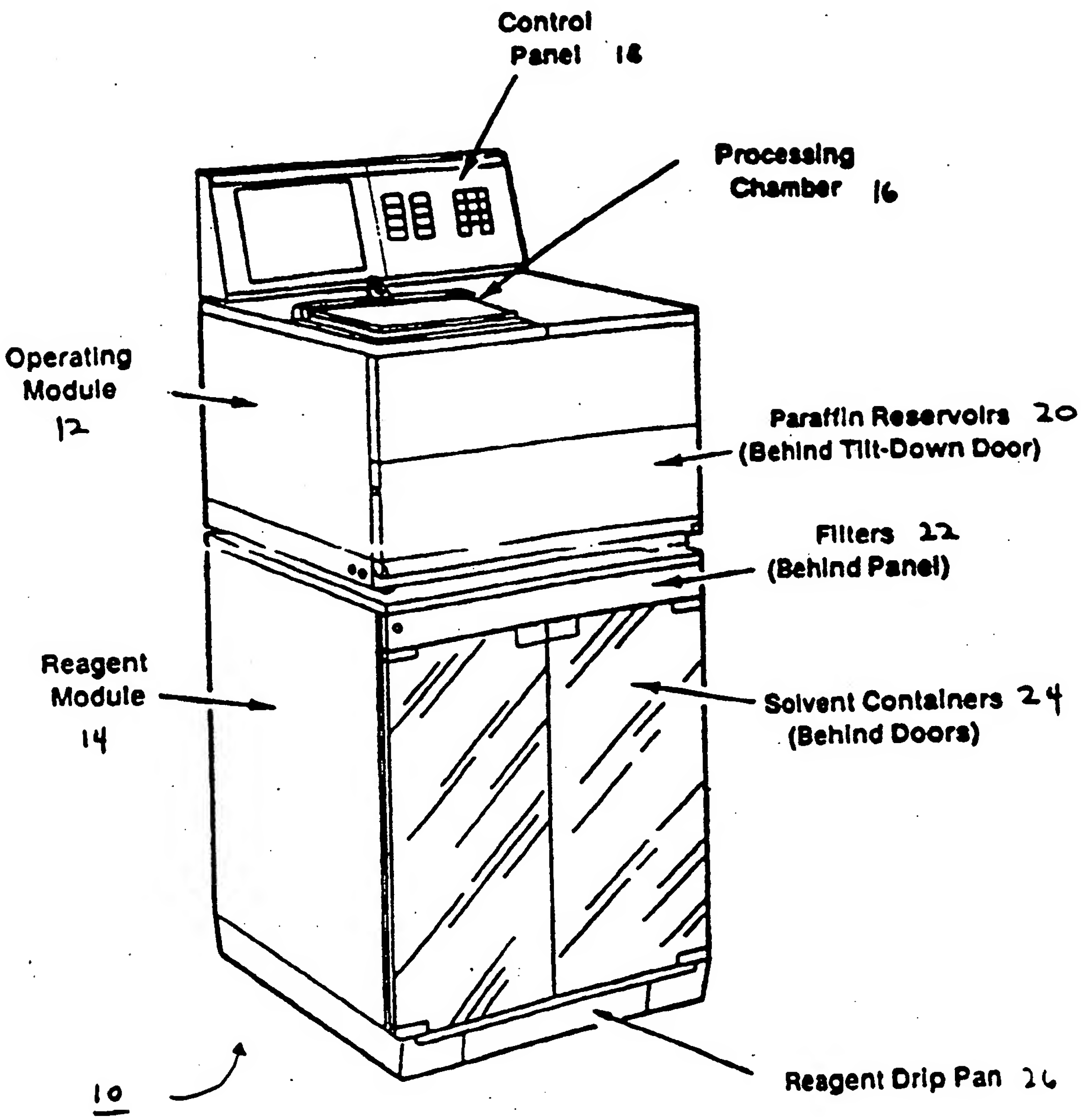

Control Panel 18
Processing Chamber 16
Operating Module 12
Paraffin Reservoirs 20
(Behind Tilt-Down Door)
Filters 22
(Behind Panel)
Reagent Module 14
Solvent Containers 24
(Behind Doors)
10
Reagent Drip Pan 26

FIGURE 1

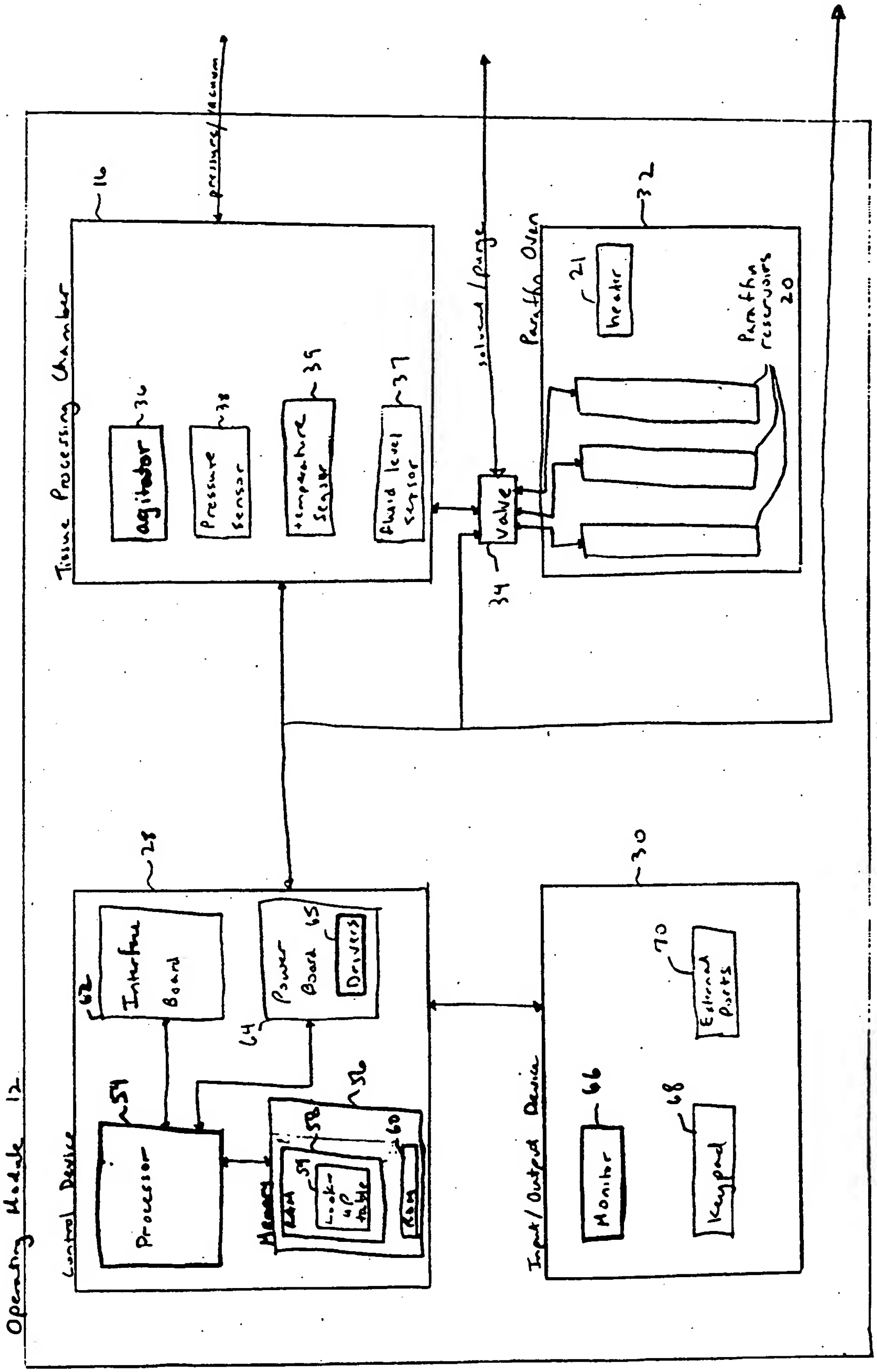

Operating Module 12
Control Device
28
Interface Board
62
Power Board
64
65
Drivers
Processor
54
Memory
56
RAM
58
59
Look-up table
ROM
60
Input/Output Device
30
Monitor
66
Keypad
68
External Ports
70
Tissue Processing Chamber
16
pressure/vacuum
agitator
36
Pressure sensor
38
temperature sensor
35
Fluid level sensor
37
solvent/purge
Valve
34
Paraffin Oven
32
heater
21
Paraffin reservoirs 20

Figure 2a

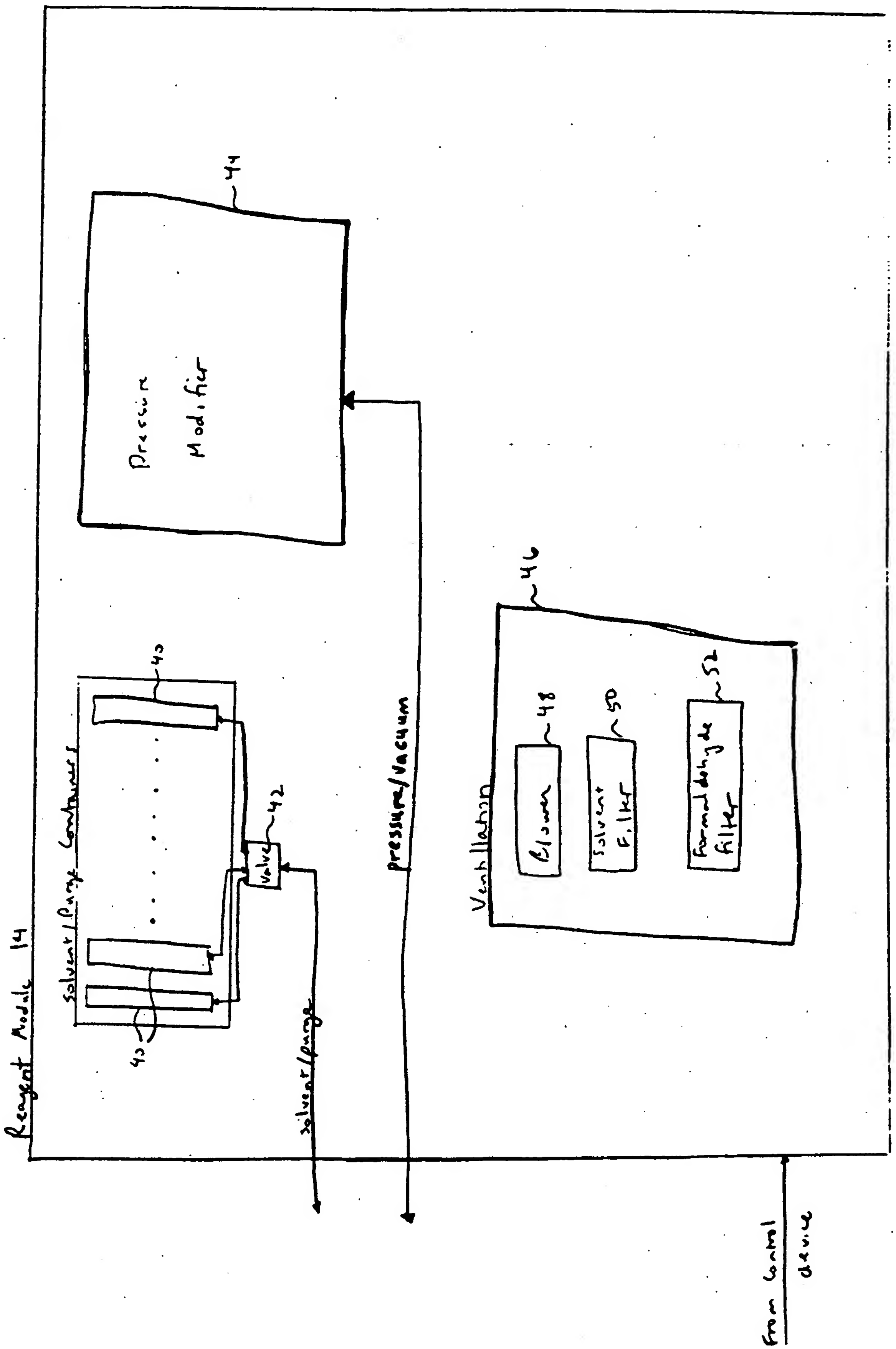

Reagent Module 14
Solvent/Purge Containers
40
40
40
Valve
42
solvent/purge
Pressure Modifier
44
pressure/vacuum
Ventilation
46
Blower
48
Solvent Filter
50
Formaldehyde filter
52
From Control device

FIGURE 2B

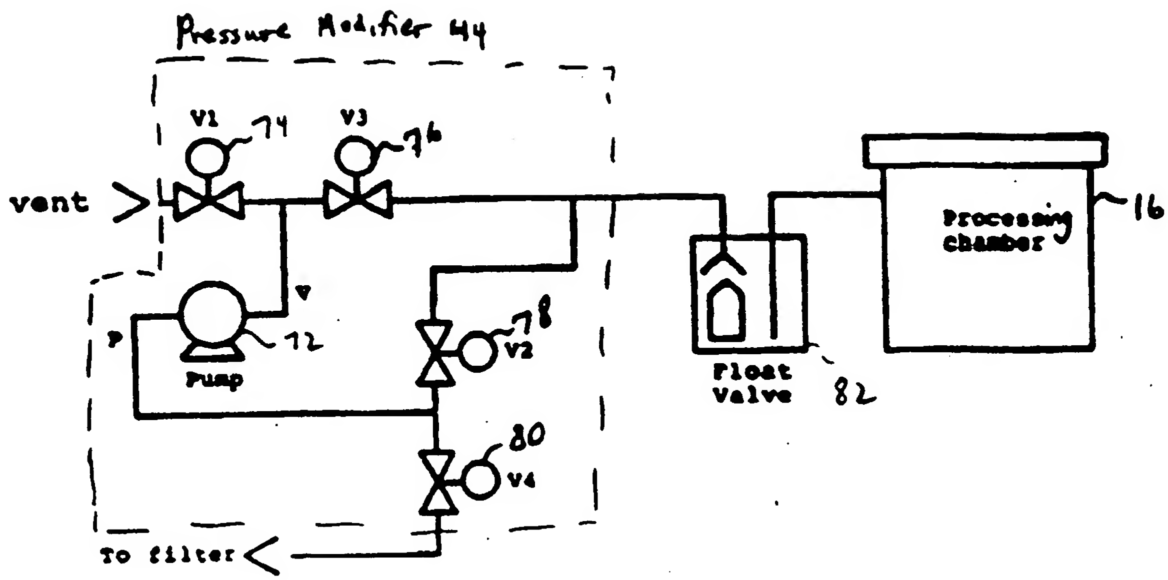
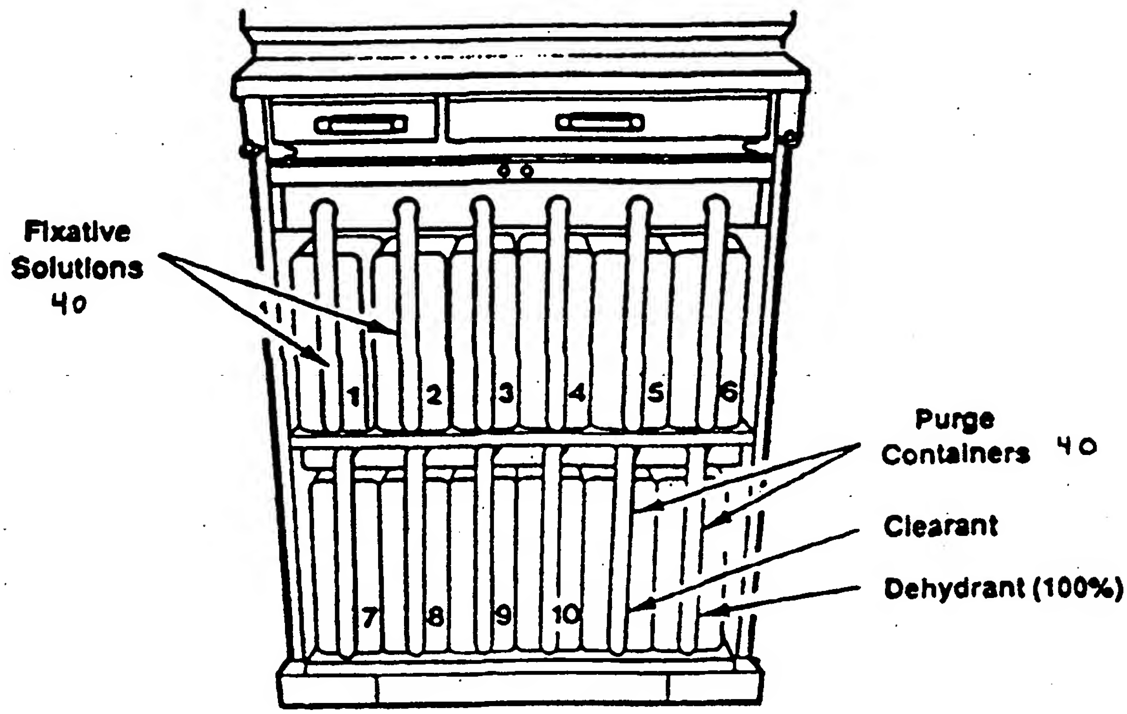

Pressure Modifier 44
V1
74
V3
76
vent
Processing chamber
16
V
P
72
Pump
78
V2
Float Valve
82
80
V4
To filter

FIGURE 3




Solvent Container Positions in the Reagent Module

FIGURE 4

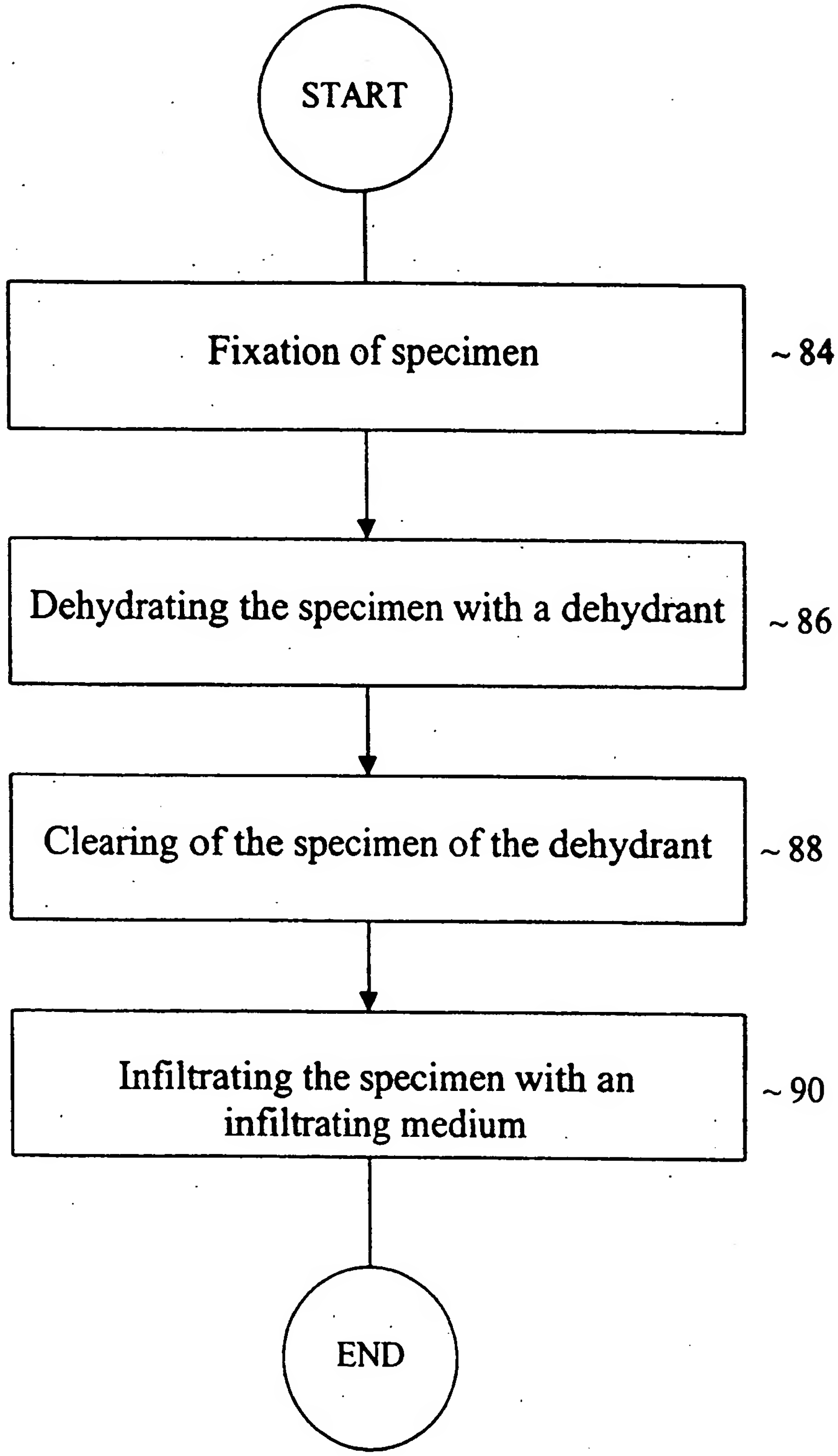

START
Fixation of specimen
~ 84
Dehydrating the specimen with a dehydrant
~ 86
Clearing of the specimen of the dehydrant
~ 88
Infiltrating the specimen with an infiltrating medium
~ 90
END

FIGURE 5

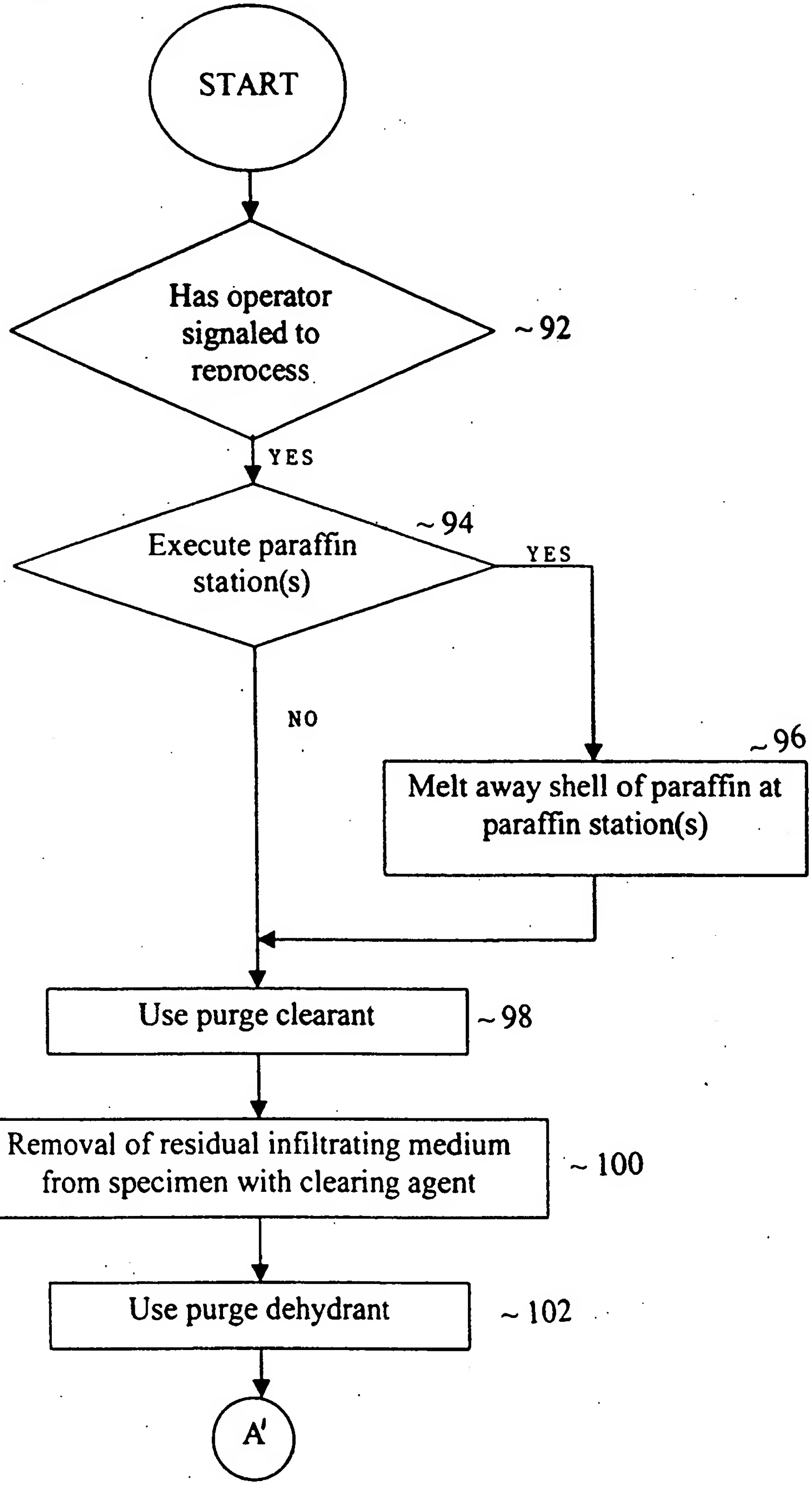
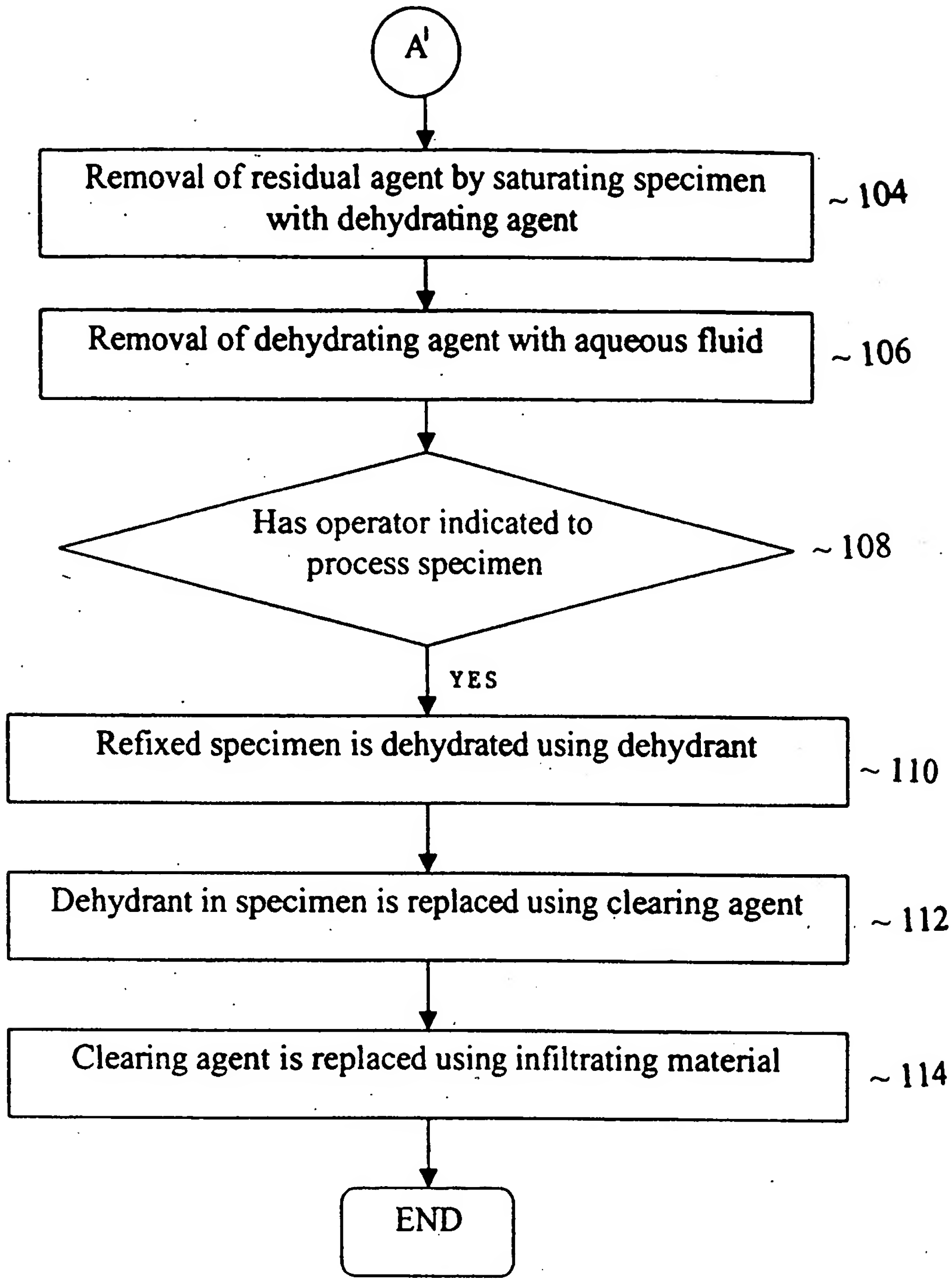

START
Has operator signaled to reprocess
92
YES
Execute paraffin station(s)
94
YES
NO
Melt away shell of paraffin at paraffin station(s)
96
Use purge clearant
98
Removal of residual infiltrating medium from specimen with clearing agent
100
Use purge dehydrant
102
A'

FIGURE 6a


A'
Removal of residual agent by saturating specimen with dehydrating agent
~ 104
Removal of dehydrating agent with aqueous fluid
~ 106
Has operator indicated to process specimen
~ 108
YES
Refixed specimen is dehydrated using dehydrant
~ 110
Dehydrant in specimen is replaced using clearing agent
~ 112
Clearing agent is replaced using infiltrating material
~ 114
END

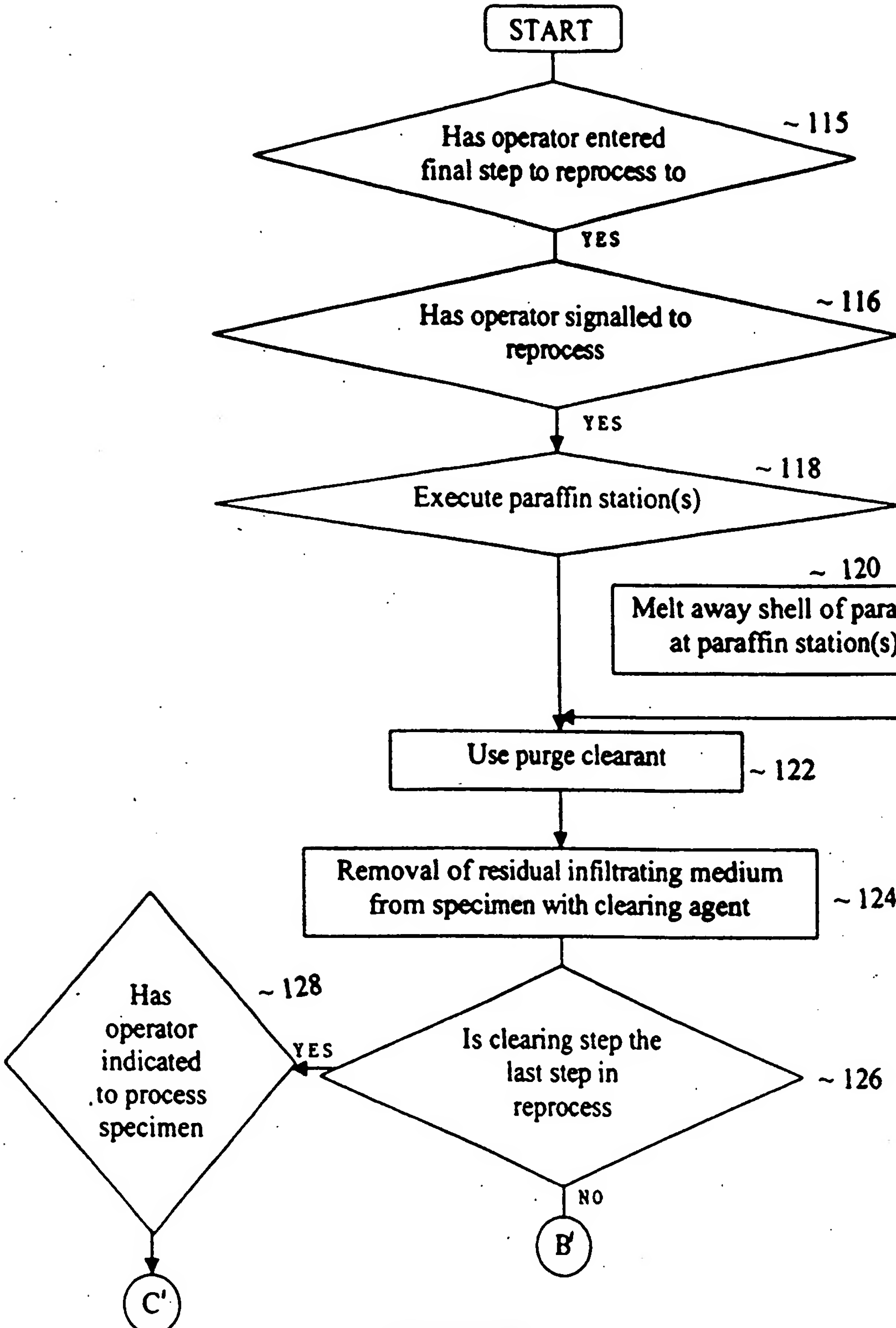
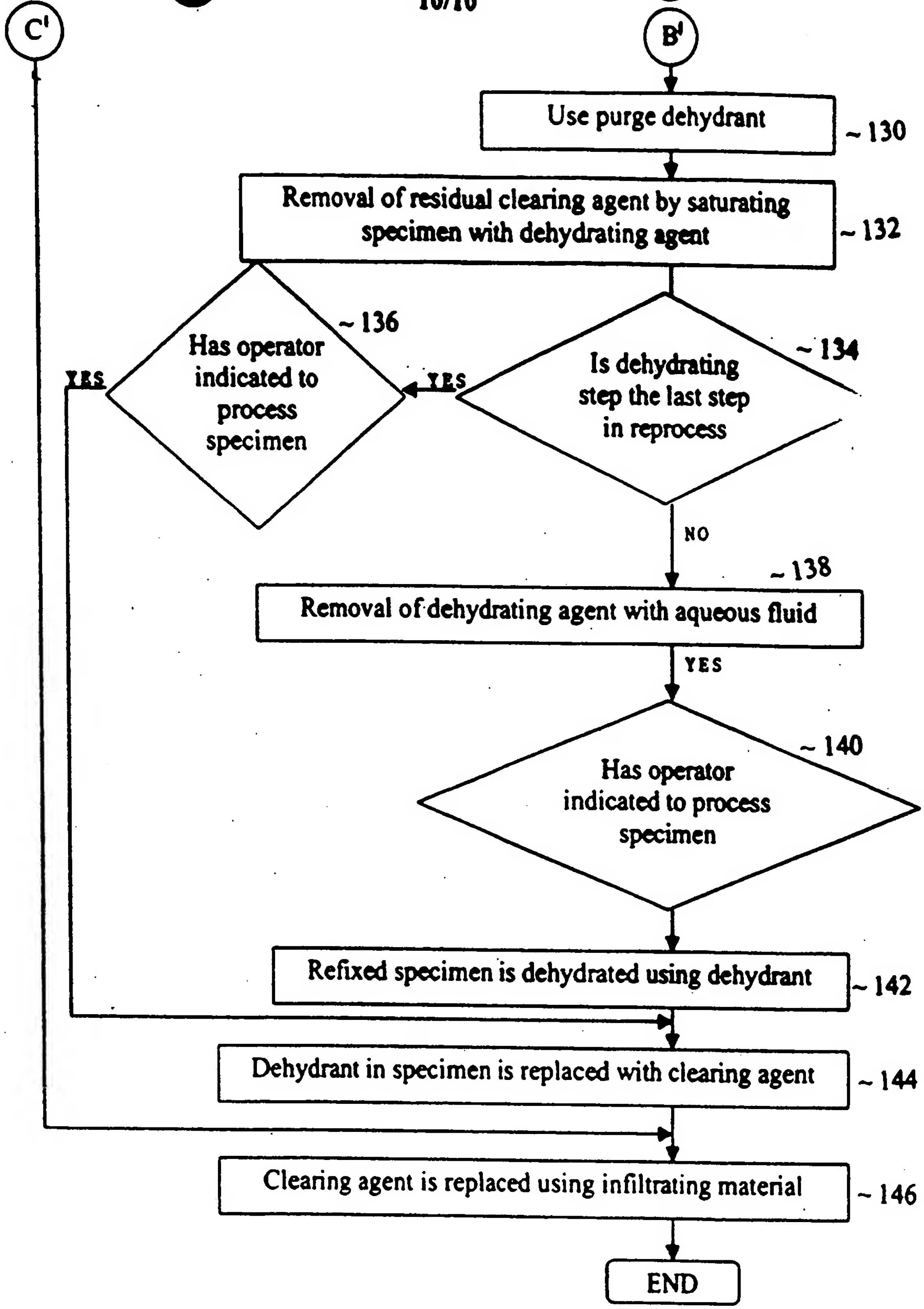

START
Has operator entered final step to reprocess to
~115
YES
Has operator signalled to reprocess
~116
YES
Execute paraffin station(s)
~118
YES
~120
Melt away shell of paraffin at paraffin station(s)
Use purge clearant
~122
Removal of residual infiltrating medium from specimen with clearing agent
~124
Has operator indicated to process specimen
~128
YES
Is clearing step the last step in reprocess
~126
NO
B'
C'

FIGURE 6b


C'
B'
Use purge dehydrant
~ 130
Removal of residual clearing agent by saturating specimen with dehydrating agent
~ 132
Has operator indicated to process specimen
~ 136
YES
YES
Is dehydrating step the last step in reprocess
~ 134
NO
~ 138
Removal of dehydrating agent with aqueous fluid
YES
Has operator indicated to process specimen
~ 140
Refixed specimen is dehydrated using dehydrant
~ 142
Dehydrant in specimen is replaced with clearing agent
~ 144
Clearing agent is replaced using infiltrating material
~ 146
END